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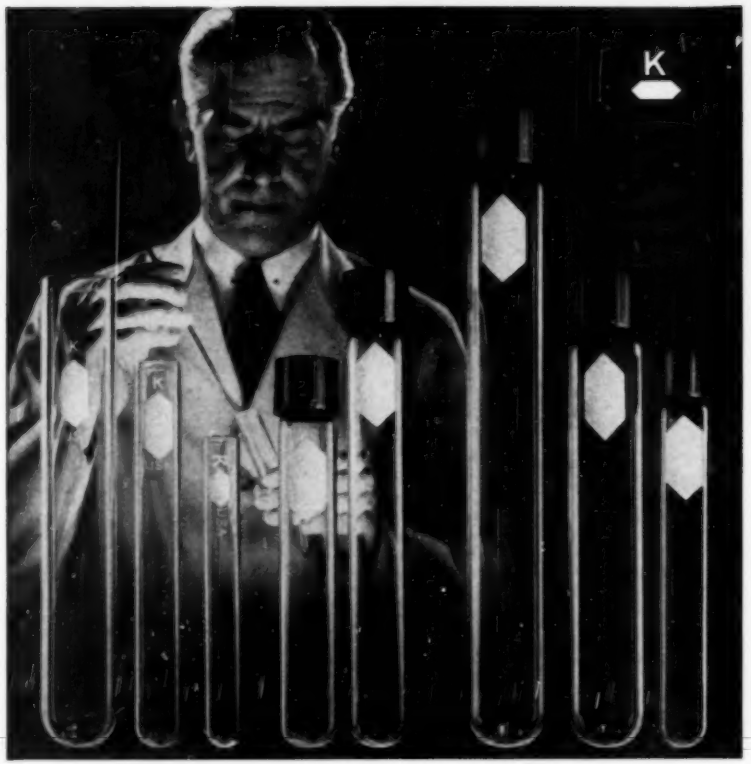

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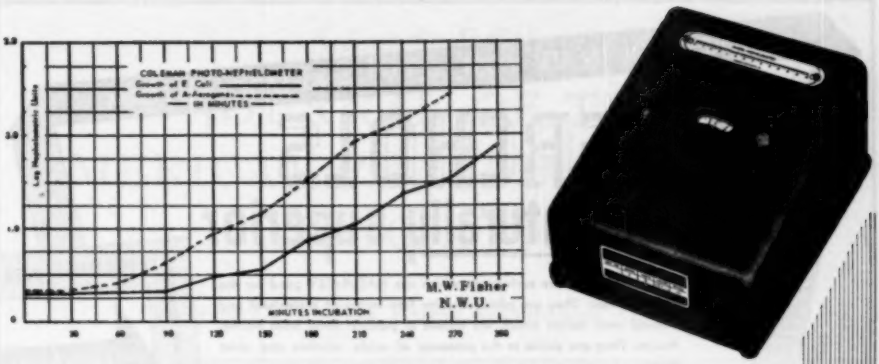

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VOLUME XXXIX — *NOVEMBER, 1950* — NUMBER 11
CONSECUTIVE NO. 22

Statistical Variation in Contents of Dry-Filled Ampuls in Current Pharmaceutical Practice*

By MELVIN W. GREEN† and LILA F. KNUDSEN‡

Three types of sampling plans applying to weights of dry-filled ampuls of a constant sample size of 20 ampuls are discussed. It is shown that a plan based on averages possesses greater discriminatory power than one based on individuals for a sample size of 20.

A NUMBER of drugs used for parenteral medication are not stable in solution. To obviate this, the manufacturer dispenses the solid drug in sterile, sealed glass ampuls or other containers in which the physician adds sterile solvents prior to injection. These preparations are usually referred to as dry-filled ampuls.

In 1945, the Canadian Government amended their Food and Drug Act (1) so as to control the weight tolerance of such dry-filled ampuls. In 1946, the National Institute of Health (2) promulgated similar tolerances for dry-filled ampuls of trivalent organic arsenical compounds. Neither agency made provision for taking a specific number of ampuls for the sample, nor did the Canadian standards indicate whether the standards were to apply to individual ampuls or averages of several. The N.I.H. standards apply to individual ampuls.

In order to measure current production of dry-filled ampuls against such standards and to provide a foundation for future standards, the project described in this paper was started in the laboratory of the AMERICAN PHARMACEUTICAL ASSOCIATION using statistical planning in order to get the most out of the data. A portion of the data has been published (3). Four arsenical drugs, procaine hydrochloride, gold and sodium thiosulfate, and a barbiturate were selected as being representative of the few drugs packaged in this manner. Five principal manufacturers' products were submitted and wherever possible 15 lots were analyzed using samples of 10 from each lot. In one case 24 lots were involved and in a few cases less than 15 lots were available. The method of securing the data has been previously described (3). Altogether 612 lots were included in the study. It is believed that the total sample is not too different from a random sample from open-market purchase. This is borne out by the fact that the results are in reasonable agreement with those previously obtained on a smaller sample (4). Preliminary

* Received June 20, 1950, from the Food and Drug Administration, Federal Security Agency, Washington 25, D. C.
Presented to the annual meeting of the American Statistical Association, December 30, 1948, Cleveland, Ohio.
† Director of the Laboratory, AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., Washington, D. C.; present address: School of Pharmacy, University of Wisconsin, Madison.
‡ Statistician, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.

observation disclosed that only about half of the lots included in the study met the proposed American tolerances, assuming these standards apply to individual ampuls. Table I shows the drugs, size of ampul, manufacturer (in code) and average fill of the samples from the lots.

There are two phases of consideration involved in setting up a foundation for future standards: (*a*) the generalized tolerance which should involve the variation between lots, and (*b*) sampling variation about the generalized tolerance.

Before these two parts of the problem are dealt with separately, it may be well to mention that there are at least three possible sampling plans that could be used in order to judge the acceptability of a given lot of dry-filled ampuls.

1. The average of 20 ampuls from a single lot shall be not less than — and not more than —.

2. No individual ampul from a sample of 20 ampuls from a lot shall be less than — or more than —.

3. The average of 20 ampuls from a single lot shall be not less than — and not more than —, *and* no individual ampul from the same sample of 20 shall be less than — or more than —.

It will be noted that in any specific sampling plan the size of the sample is given (here taken as 20 for practical reasons), and also a specification that either the average net weight of a sample or the fill of any individual ampul is to be considered. Of course, there are other possible types of sampling plans than these. No matter what sampling plan is used, it must be realized that the sample rarely reflects exactly the characteristics of the lot and that two samples from the same lot will rarely agree exactly. The

TABLE I.—VARIATION IN WEIGHT OF DRY-FILLED AMPULS OF CERTAIN DRUGS

Drug	Dose, Mg.	Mfgr.	Found Average, Mg.	Corr. Std. Error of Av., $\sigma_{\bar{x}w}$	Variance Components σ_w^2	Variance Components σ_b^2
Trivalent Arsenical I	150	O	144.3	1.08	16.93	6.51
Trivalent Arsenical I (14)[a]	150	C	145.4	1.81	64.08	1.64
Trivalent Arsenical I (13)	150	T	145.1	0.96	17.67	0.88
Trivalent Arsenical I	300	C	290.1	1.97	46.21	31.35
Trivalent Arsenical I	300	T	294.7	1.53	17.67	28.99
Trivalent Arsenical I	450	C	443.5	2.30	103.97	2.26
Trivalent Arsenical I (8)	450	I	447.6	3.49	219.12	23.85
Trivalent Arsenical I	450	T	447.9	1.32	17.67	17.00
Trivalent Arsenical I	600	O	587.5	5.10	506.02	14.70
Trivalent Arsenical I (14)	600	C	592.0	2.56	86.53	40.44
Trivalent Arsenical I	600	T	596.8	1.33	25.99	9.38
Trivalent Arsenical I	750	C	737.4	2.64	94.18	45.48
Trivalent Arsenical I	750	T	745.6	1.20	15.14	13.78
Trivalent Arsenical I	900	I	893.0	2.67	134.16	7.90
Trivalent Arsenical I (10)	900	T	893.9	1.56	24.20	24.24
Trivalent Arsenical II	100	C	96.6	1.72	59.17	0.12
Trivalent Arsenical II	200	C	197.8	1.93	65.52	8.98
Trivalent Arsenical II	300	C	294.6	1.68	56.45	0
Trivalent Arsenical II	400	C	395.9	2.84	161.31	0
Trivalent Arsenical II (14)	500	C	497.8	1.68	53.14	3.12
Trivalent Arsenical III (24)	300	K	308.2	0.97	10.37	8.45
Trivalent Arsenical IV	290.4	I	294.8	2.14	64.08	27.85
Procaine Hydrochloride	50	O	49.98	0.32	0.97	1.05
Procaine Hydrochloride	50	T	48.50	0.59	5.20	1.65
Procaine Hydrochloride	100	O	100.0	0.63	5.76	2.21
Procaine Hydrochloride (12)	100	I	99.9	0.90	14.45	1.68
Procaine Hydrochloride	100	T	98.9	0.88	12.17	3.23
Procaine Hydrochloride	120	T	118.0	0.88	12.80	2.55
Procaine Hydrochloride	150	T	148.2	1.17	19.21	8.14
Procaine Hydrochloride (9)	200	I	199.4	1.14	25.99	0.018
Procaine Hydrochloride	200	T	198.5	1.15	21.22	5.10
Procaine Hydrochloride (8)	500	T	498.0	1.45	22.05	20.16
Gold and Sodium Thiosulfate	10	O	9.68	0.23	0.80	0.21
Gold and Sodium Thiosulfate	10	C	9.60	0.24	1.46	0
Gold and Sodium Thiosulfate	25	O	24.3	0.41	1.68	1.66
Gold and Sodium Thiosulfate	25	C	23.8	0.40	3.20	0.04
Gold and Sodium Thiosulfate (14)	50	O	49.8	0.41	2.59	0.69
Gold and Sodium Thiosulfate (14)	50	C	48.9	0.36	2.59	0
Gold and Sodium Thiosulfate (11)	100	O	99.7	0.49	4.42	2.46
Gold and Sodium Thiosulfate (13)	100	C	98.5	1.05	7.20	15.00
Barbiturate I	1,000	T	989.0	4.81	492.03	0

[a] Numbers in parentheses indicate number of lots examined; all other cases, 15 lots examined.

amount of variation between samples and the comparison between the lot and a sample from the lot can be predicted by means of some of the statistical techniques developed in recent years.

STATISTICAL TECHNIQUES

The Generalized Tolerance

Generalized tolerances or definitions of acceptable lots of drugs are often set administratively, taking into account the economics involved, and technical considerations or medical viewpoint as to the variation permissible from lot to lot. However, the data given in Table II could form an excellent basis for such tolerances by using some multiple of the variation between lots obtained by statistical analysis.

A brief explanation of the statistical calculations may be helpful. The measure of variation between lots used here was calculated by considering the within-lot variance component as that of random error; adding together the variance component between lots and that within lots and performing the proper arithmetical computations in order to obtain the "corrected" standard error of the average which we label as $\sigma_{\bar{x}\bar{w}}$. A sample calculation is shown below, including the table for the analysis of variance for results on 100-mg. ampuls of procaine-hydrochloride made by manufacturer "O." Assume that the sampling plan requires that the average net weight of a sample of 20 ampuls from a lot be above the generalized tolerance.

There are 15 lots of 10 ampuls each, making 150 ampuls altogether. An explanation of analysis of variance can be found in almost any statistical text (5).

	Degrees of Freedom	Sum of Squares	Mean Square
Between lots	14	390.56	27.90
Within lots	135	777.60	5.76
Total	149	1,168.16	

Variance Components

$$\text{Between lots} = \frac{27.90 - 5.76}{10} = 2.21 = \sigma_B^2$$

$$\text{Within lots} = 5.76 = \sigma_W^2$$

"Corrected" standard error of averages[1] of 20 $= \sqrt{\frac{\sigma_W^2 + \sigma_B^2}{N}} = \sqrt{\frac{2.21 + 5.76}{20}}$

"Corrected" standard error of averages[1] of 20 $= \sigma_{\bar{x}\bar{w}} = 0.63$

[1] Note that this is the same as the standard error of the average calculated for taking one ampul from each of 20 lots, and contrast it with the following calculation which is for taking 20 ampuls from one lot.

Corrected standard error of averages of 20 $= \sqrt{\sigma_B^2 + \frac{\sigma_W^2}{N}} = \sqrt{2.21 + \frac{5.76}{20}} = 1.58$

In other words, the expected variation around the labeled weight would be less if 20 ampuls were taken one from each of 20 lots than the variation around the labeled weight of 20 ampuls taken from the same lot. This is because in the second instance only one setting of the filling machine is involved which would naturally vary more from the labeled weight than an average of 20 settings of the filling machine.

TABLE II.—CRITERIA FOR LIMITS FOR DRY-FILLED AMPULS BASED ON AVERAGES AND ON INDIVIDUALS

Wt. Ranges, Mg.	Limits Based on Averages	Limits Based on Individuals
Less than 26	±10%	±45%
26–50	±6.0%	±25%
51–150	±6.0%	±25%
151–500	±10 mg.[a]	±45 mg.
More than 500	±10 mg.[a]	±45 mg.

[a] 10 mg. was selected rather than 7.5 mg. in order to include more of the data from Fig. 1.

Table I also shows the corrected standard errors of averages of 20 which are labeled $\sigma_{\bar{x}\bar{w}}$ and the variance components from which they were obtained. Figure 1 shows these values of $\sigma_{\bar{x}\bar{w}}$ plotted against the labeled dose with the manufacturer indicated by code letter. It must be acknowledged that some of these corrected standard errors are too high and some too low just from sampling fluctuation. In fact, the accuracy of the computed variances could be estimated (6). However, for the purpose of illustration here, freehand lines were drawn on the chart such that the majority of manufacturers, kinds of drugs, and sizes of ampuls fell below the line. Such lines have been drawn on Fig. 1 so that below 125 mg. the value of $\sigma_{\bar{x}\bar{w}}$ is 2% of the labeled dose as indicated by the slanting line. For doses above 125 mg. a horizontal line drawn at $\sigma_{\bar{x}\bar{w}} = 2.5$ mg. appears to include most of the data, excepting a few close to the line and a few which are obviously way out of line.

Current usage and policy have accepted the 3-σ limit as indicated by the Shewhart phrase "it works," although some manufacturers and statisticians are of the opinion that 3-σ limits are too wide and that 2-σ limits are better. For purposes of comparison and illustration, 3-σ limits will be used here. Taking 3 $\sigma_{\bar{x}\bar{w}}$ from the freehand line on the chart, the illustrative generalized tolerance for ampuls below 125 mg. would be three times 2%, or 6%. (Thus upper and lower tolerance limits would be 94% and 106%, respectively.) Again trying the 3-σ limit for ampuls above 125 mg., one would calculate three times 2.5 mg., or 7.5 mg., as a comparative limit.

Now let us compare these data with the implications involved in the various proposed or promulgated tolerances mentioned beforehand. We do not wish to advocate, specify, or criticize tolerances, but merely compare the variations found with the variations implied in these aforementioned tolerances. In the case of the Canadian tolerances and those proposed by a group of manufacturers, we have taken the liberty of recalculating the tolerances so that they will be based on averages of 20 to make them comparable to the calculations based on our findings. When these adjusted tolerances are plotted as in Fig. 2, it can be seen that in certain areas, notably at the lower weights, tolerances tend to be narrow, while at higher weights they tend to be broad when a sliding scale of tolerances is used.

In connection with determining tolerance limits, there are various methods besides the simple 3-σ Shewhart "it works" limits. Tolerance limits may indicate the range of normal variation in various

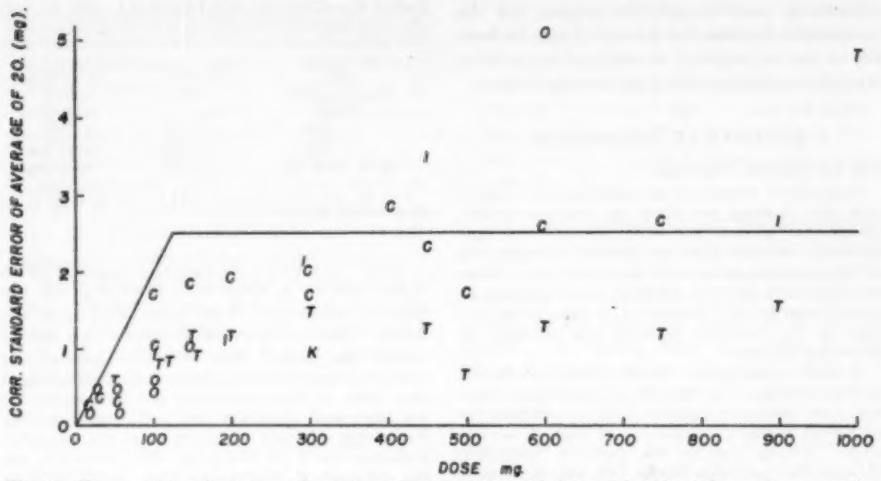


Fig. 1.—Corrected standard error of averages plotted against labeled weight (dose) for various manufacturers. Solid line is a suggested maximum.

characteristics or the quality bounds within which a certain percentage of a manufactured product may be expected to occur. Some of the newest ideas on this subject are expounded in Chapter II of the Columbia Research Group's "Selected Techniques of Statistical Analysis" (7). Here they give methods for obtaining tolerance limits from estimates of the mean and standard deviation obtained from a sample of N observations such that in a fraction γ of the samples from which they are computed, the limits will include at least a fraction P of the items of the distribution.

Sampling Variation about the Generalized Tolerance (Operating Characteristic Curves)

Suppose that the generalized tolerance is given or has been calculated, then the second phase of consideration concerns the effect of the sampling

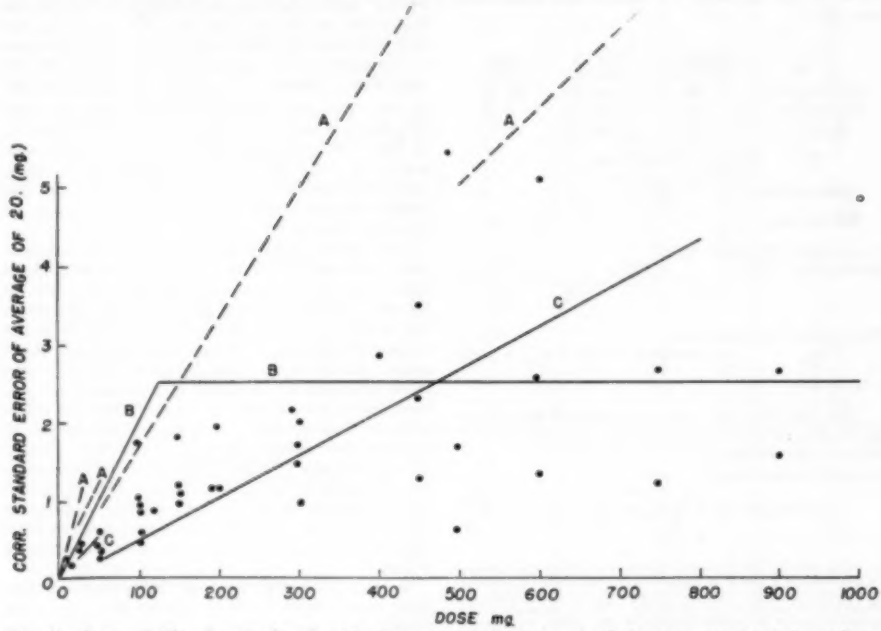


Fig. 2.—Data of Fig. 1 reproduced with tolerances indicated. *A*, Tolerance suggested by mfgrs.; *B*, Tolerance suggested by data; *C*, Canadian tolerance, sample 10.

variation on whether or not a specific lot is accepted when a certain sampling plan is used. As stated before, the examination of a sample from a lot will rarely give exactly the same results as an examination of the entire lot; and that even successive samples from the same lot will give varying results. How much these results will vary due to chance alone can be calculated statistically and forms the basis for the construction of "operating characteristic curves."

Given a particular sampling plan, a tolerance limit and a lot whose actual average fill and variation in fill are known, it is possible to determine what percentage of samples would yield average fills above the specified tolerance. Therefore, it is possible to calculate the probability of passing a lot of given quality. By "actual average fill" is meant that obtained if every single ampul in the lot were examined. Suppose we take a sampling plan for 100-mg. ampuls of procaine hydrochloride, which states that in a sample of 20 from the lot, the average fill should not be less than 94 mg. nor more than 106 mg. If the standard deviation within the lot were known, it is possible to calculate what percentage of the time a *lot* having an actual average fill of 95 mg. would yield samples of 20 whose average fills would be above 94 mg. This would be the probability of that lot being accepted. Similar calculations can be made for lots having actual average fills of 96 mg., 93 mg., 92 mg., etc. Given a lot having an actual average exactly at the tolerance limit (here 94 mg.), and drawing a sample of 20 ampuls from that lot, half of the time the sample will have an average above the tolerance and half of the time below the tolerance. These calculations[2] have been made on the probability of accepting lots of ampuls, having various average fills. A chart of these probabilities plotted against the specific average value of the lot is called an operating characteristic (*OC*) curve.

The *OC* curve for this sampling plan, as applied to ampuls labeled to contain 100 mg. of procaine hydrochloride (manufacturer O), is shown in Fig. 3. On this curve the vertical dotted lines represent generalized tolerance limits. In this particular case, the limits have been set both above and below the labeled weight at ±6 mg., which is three times $\sigma_{\bar{x}}$. The curves are so calculated that the probability of accepting a lot having an average equal to the generalized tolerance limit is 50%.

Suppose the following is a specification for 100-mg. dry-filled ampuls:

> A sample of 20 ampuls is to be selected at random from a lot and the average weight of the contents determined. If the average is not less than 94 mg. nor more than 106 mg. the lot is to be accepted. If the average falls beyond these limits, the lot is to be rejected.

From the curve in Fig. 3 it can be seen that if the average of the lot were actually the labeled quantity (100 mg.) the probability of acceptance, P_a, would be 100%, as it should be. It is of interest to note that, about 5% of the time, or one time in 20, a lot would be accepted whose average is actually 93.1 mg. Also 5% of the time a lot would be accepted whose average is 106.9 mg. If the lot average is 94 mg. or 106 mg., the lot will be accepted 50% of the time.

Figure 4 shows the same thing as the previous curve except that only half of the curve is plotted (i.e., deviation from only one limit marked 0.0), and it also shows another *OC* curve calculated for the same size ampul of the same drug manufactured by another firm (I). (These curves are based on a sampling plan involving averages of 20 ampuls.) The *OC* curve with the steeper slope is associated with the smaller variation within lots. It can be seen from Table I that the variance component within lots for 100-mg. ampuls of procaine hydrochloride for manufacturers I and O are 14.45 and 5.76, respectively. These two *OC* curves can be compared to show that if a lot had an average fill which was 1 mg. below the generalized tolerance, the chances of passing the lot would be 12 in 100 if it had been made by manufacturer I, and 3 in 100 if it had been filled by manufacturer O. Similarly, if a lot had an average fill which was 1 mg. *above* the generalized tolerance, the chances of passing the lot are 88 in 100 for manufacturer I, and 97 in 100 for manufacturer O. This latter statement, of course, can also be stated in another way: the chances of rejecting the lot are only 3 in 100 for manufacturer O, while they are 12 in 100 for manufacturer I. Thus it is possible to determine the chances taken of accepting a "bad" lot (one whose average is actually below the generalized tolerance) and rejecting a "good" lot (one whose average is actually above the generalized tolerance).

Figure 5 shows *OC* curves for gold and sodium thiosulfate in 10-mg. ampuls as made by two manufacturers, O and C, assuming the same type of sampling plan is used.

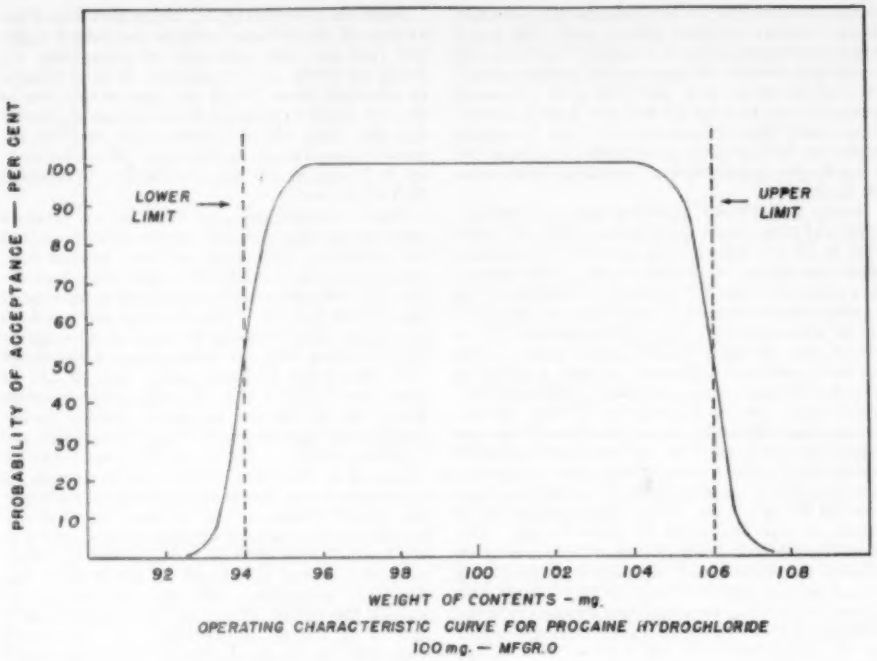
Figure 6 is of interest because it represents *OC* curves for two different drugs by different manufacturers, namely, manufacturers K and C, at the 300-mg. level, with the same type of sampling plan based on the average of 20 ampuls from a lot. The solid line represents the drug which appeared to exhibit the best manufacturing practice (smallest variance component within lots) of all of the drugs studied at this size of ampul. It can be noted that this curve is much the steeper of the two. The probability of accepting a lot whose average is actually 2 mg. below the standard is practically zero for manufacturer K, arsenical III, but is about 10 out of 100 for manufacturer C, arsenical I.

Figure 7 shows the *OC* curves for weight fills of trivalent arsenical I of manufacturers O and T at the 600-mg. level. The curve for manufacturer O is very flat. It can be noted from Fig. 1 that this is one of the products which was clearly out of line as far as grand average and corrected standard error of the average are concerned. The probability of accepting a lot whose average is 3 mg. below the tolerance is practically zero for manufacturer T while it is 27 out of 100 for manufacturer O.

[2] Given the tolerance limit L for averages of 20, and assuming normality, the probability of acceptance of a lot with average m and the within-lot standard deviation σ is given by

$$P_a = \int^{\infty} \frac{(L - m)}{(\sigma/\sqrt{n})} \phi(t)dt$$

where $\phi(t) = (1/\sqrt{2\pi})e^{-t^2/2}$ which is the normal curve of error. To calculate this probability, it is necessary to transform the various distances around the set limit into units of the standard error of the average and then by means of normal distribution tables (one tail) transform these values into probabilities of acceptance.



OPERATING CHARACTERISTIC CURVE FOR PROCAINE HYDROCHLORIDE 100 mg. — MFGR. O

Figure 3.

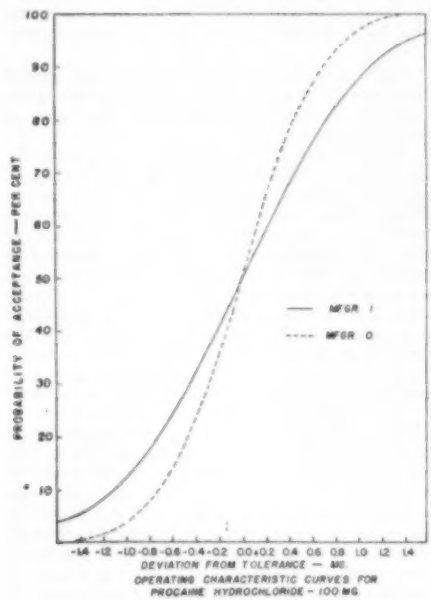

PROBABILITY OF ACCEPTANCE — PER CENT
MFGR. I
MFGR. O
-1.4 -1.2 -1.0 -0.8 -0.6 -0.4 -0.2 0.0 +0.2 0.4 0.6 0.8 1.0 1.2 1.4
DEVIATION FROM TOLERANCE — MG.
OPERATING CHARACTERISTIC CURVES FOR PROCAINE HYDROCHLORIDE - 100 MG.

Figure 4.

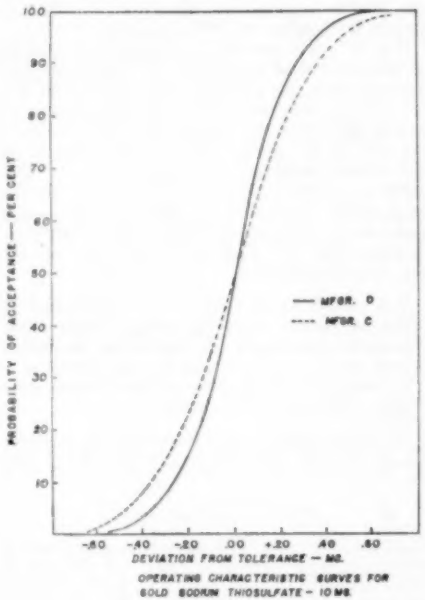

PROBABILITY OF ACCEPTANCE — PER CENT
MFGR. D
MFGR. C
-.60 -.40 -.20 .00 +.20 .40 .60
DEVIATION FROM TOLERANCE — MG.
OPERATING CHARACTERISTIC CURVES FOR GOLD SODIUM THIOSULFATE - 10 MG.

Figure 5.

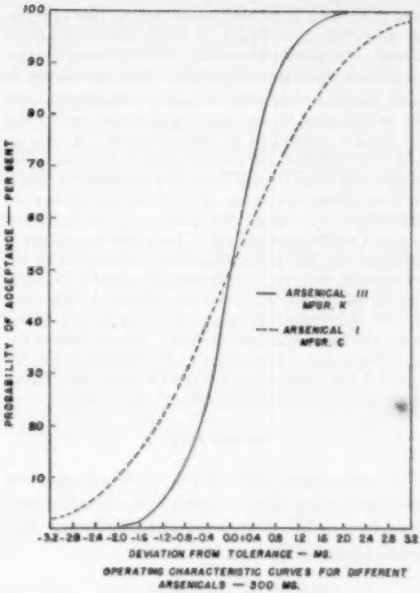


Figure 6.

It must be remembered that these *OC* curves shown in Figs. 3 to 7 are the operating characteristic curves of the various manufacturers' processes as represented by the samples and the resulting index of variation in the manufacturing process. Thus, as indicated in Fig. 7, manufacturer O may be using the same sampling plan for inspection as manufacturer T, but yet a lot of the material O puts out will be of poorer quality than that of manufacturer T because of the wide variation in his filling practices. It follows that it is highly desirable to reduce the variation in a manufacturer's filling practices as much as possible. The use of control charts and other methods of checking the statistical stability of the variances is almost imperative.

Considering Sampling Plans Based on a Tolerance for Individual Ampuls

The previous discussion and *OC* curves shown in Figs. 3 through 7 have been based on sampling plans specifying that the average weight of 20 ampuls taken from a lot must be between certain limits. The question has been raised as to what would be the appearance of the *OC* curve for a sampling plan specifying that a sample of 20 ampuls be taken from a lot and the lot rejected if a single ampul is below some specified tolerance. Such an *OC* curve is constructed in a similar manner[3] and is illustrated by the dotted line on Fig. 8, which is based on data for weight of 100-mg. ampuls of procaine hydrochloride.

The sampling plan used stated that the weight of no individual ampul of a sample of 20 ampuls can fall below 89.6. (This arbitrary limit was used in order to obtain an *OC* curve which would coincide with the *OC* curve for averages at the 50% point.) The *OC* curve for the same data, but using a sam-

[3] If it is required that a specified number $(n - k)$ of items of a sample of n exceed the limit L', the probability of acceptance for this plan, assuming a normal population, is

$$P'_a = \sum_{r=0}^{k} \frac{n!}{(n-r)!r!} p^{n-r}(1-p)^r$$

where

$$p = \int_{\frac{(L'-m)}{\sigma}}^{\infty} \phi(t)\,dt$$

and as before $\phi(t)$ is the normal curve of error; m = average of the lot; σ = within lot standard deviation; r = the number of defective ampuls, r summed from $r = 0$ to k, where k is the maximum number of allowable defectives. In our case we are allowing no defective units; therefore, $r = k = 0$ and the formula P'_a reduces to $(p)^n$.

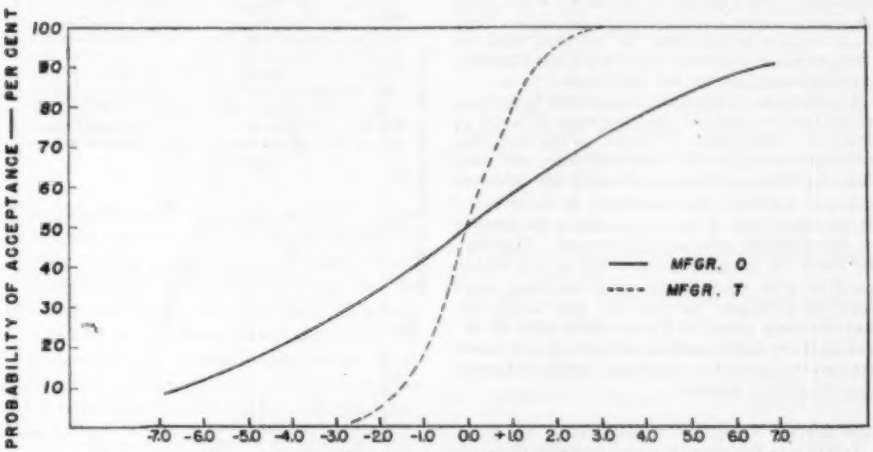


Figure 7.

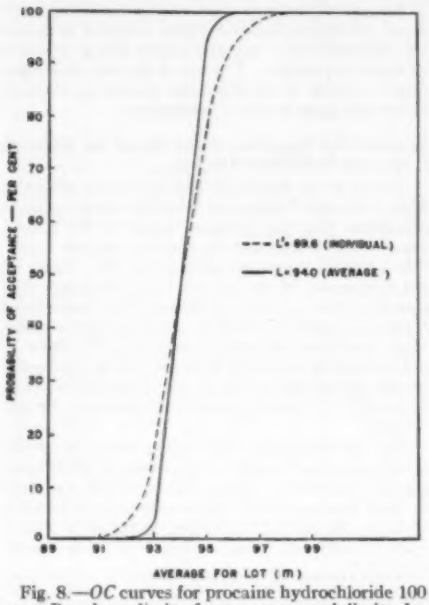


Fig. 8.—*OC* curves for procaine hydrochloride 100 mg. Based on limits for averages and limits for individuals.

pling plan based on the average weight of a sample of 20 ampuls, is shown by the solid line in Fig. 8. Comparison of the two sampling plans directly is possible since each shows a 50% probability of accepting lots whose average fill is 94 mg. The probability of accepting a lot whose actual average was 93 mg. is 3 out of 100 for the plan based on averages, and 17 out of 100 for the plan based on individuals. The slope of the *OC* curve for the average plan is steeper than that for individuals. Thus it can readily be seen that the sampling plan for averages has greater discriminatory power than the attribute sampling plan for individuals.

A comparison of the limits that would have to be set for the two types of sampling plans is shown in Table II. Both limits are based on the corrected standard deviations as calculated from the data shown in Table I; however, of course the corrected standard deviation for individuals is $\sqrt{20}$ times the standard error of the average (from the formula for the standard error of the average). Therefore the limits for sampling plans based on individuals would be $\sqrt{20}$ times the limits for sampling plans based on averages. So that not only would the discriminatory power of the sampling plan be decreased if the plan based on individuals were used, but also the generalized tolerance would be farther from the labeled amount.

Considering Sampling Plans Based on Joint Tolerances for Averages and Individuals

The *OC* curve for this type of sampling plan was approximated in a different manner from the preceding figures. Two hundred random samples of 20 observations each, drawn from a normal population (8,9) were examined according to two criteria: (*a*) a specification based on limits for averages of 20 and (*b*) a specification based on limits for averages as well as limits for individuals where this latter is $\sqrt{20}$ times the limit for averages. Figure 9 shows the comparison of the two approximations to the *OC* curves arrived at in this manner. It can be seen that the two curves differ very little in selectivity, and that in this case the addition of limits on individuals to the use of limits on averages does not effectively change the percentage of defective lots that would be accepted. The relative amount of influence of the *OC* curves for averages and for individuals on the composite *OC* curve depends on the difference between the limits L (for av.) and L' (for indiv.) in terms of the standard deviation. If this difference is large, the *OC* curve for individuals has little effect. An approach to this problem has been discussed by Dr. John W. Tukey in an unpublished memorandum report (10).

SUMMARY

In connection with setting a foundation for possible standards for weights of dry-filled ampuls of certain drugs, it is necessary to consider two definite parts of the problem:

1. A generalized tolerance—this can be derived from the variation between lots, as arrived at by means of variance components.

2. The sampling variation involved in arriving at a decision about lots of certain definite qual-

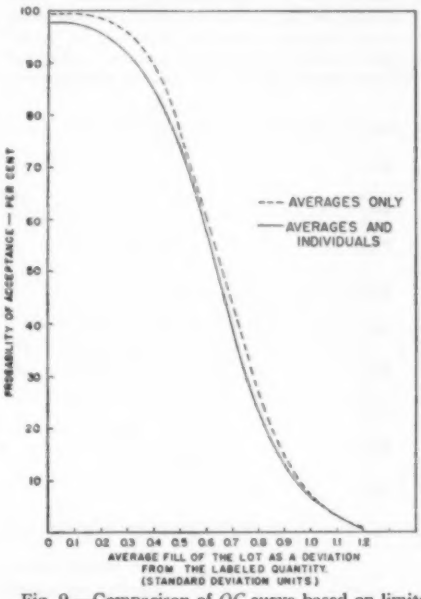

PROBABILITY OF ACCEPTANCE — PER CENT
AVERAGES ONLY
AVERAGES AND INDIVIDUALS
AVERAGE FILL OF THE LOT AS A DEVIATION FROM THE LABELED QUANTITY (STANDARD DEVIATION UNITS)

Fig. 9.—Comparison of *OC* curve based on limits for averages and individuals with *OC* curve based on averages only.

ity—this can be seen from the construction of "operating characteristic curves."

There are three types of sampling plans discussed as applying to weights of dry-filled ampuls with a constant sample size of 20 ampuls; first, that plan based on the average weight of 20 ampuls being within the generalized tolerance; second, a plan which requires that all individual ampuls be within certain limits; and third, a plan which is a combination of the first two. It is shown that the plan based on averages possesses greater discriminatory power than that based on individuals for a sample size of 20, and that even when the two plans are combined there is a negligible improvement over the use of the plan based on averages alone.

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A Study of an *in Vivo* Method of Testing Enteric Coatings Using Radioactive Tracer Techniques*

By C. F. PETERSON,† C. O. LEE, and J. E. CHRISTIAN

An *in vivo* method for testing enteric coatings is described which involves coating small pills containing radiosodium, administering them to a rat, and determining the time of disintegration by the appearance of radiosodium in the circulation and the site of disintegration by direct observation of the gastrointestinal tract. The results of the method have been compared to the results of an *in vitro* method.

THE development of a reliable method to test enteric coatings has not been completely realized. The *in vitro* methods are easy and less expensive than the *in vivo* tests but have met the objections that they do not simulate the actual conditions of the gastrointestinal tract (1–3). Of the *in vivo* methods, the one which has been most widely used is the technique of following the coated radio-opaque medicament with the fluoroscope or X-ray photographs to determine the site and the time of disintegration.

The use of the radioactive tracer technique was first suggested by the work of Lark-Horovitz and Leng (4) who administered an enteric-coated capsule containing radioactive sodium chloride. The time of disintegration was determined by the appearance of radioactivity in the circulation of the hand as measured by a counter tube shielded from the rest of the body. The exact site of disintegration cannot be established by the method described.

In this work, enteric-coated pills containing radioactive sodium (Na^{24}) chloride were administered to rats and the time of disintegration determined by the appearance of radioactivity in the circulation. The site of disintegration was established by direct examination of the gastrointestinal tract.

The sodium ion has been shown by Hamilton (5) to be quickly absorbed from the gastrointestinal tract. Sodium-24 has radiations of

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sufficient intensity to be readily detected and the half-life (14.8 hours) is short, which eliminates the danger of long term contamination (6).

EXPERIMENTAL PROCEDURE

Radioactive sodium-24[1] as sodium chloride was used to make 12-mg. pills which were about 2.5 mm. in diameter. It was necessary to make the pills at least this small so that they would pass readily into the stomach and in turn pass through the pyloric sphincter. A small amount of kaolin, acacia powder, and starch paste was used to form the mass. Gentian violet was also included so the pill remains could be easily identified. A pill cutter was devised so that the pill pipe could be cut into regular 2.5-mm. segments to make uniform pills. Rubber gloves were worn during this process to prevent contamination since the pill mass had to be handled and rolled into pills by hand. The pills were dried, sealed with a light sugar coat, and then mixed with a large number of red colored sugar granules of approximately the same size. The enteric coating formula (castor oil and shellac) proposed by Goorley and Lee (7) was applied by successively spraying the solution and drying the pills as they were rotated in a small laboratory coating pan. A protective sugar coating was applied when the enteric coating was satisfactory.

The radioactive pills were readily separated from the sugar granules since the red color of the granules was apparent through the coatings. A radioactive pill was dropped into the rear of the mouth of the rat and forced into the stomach with the blunt tip of a soft rubber catheter. The rat was immediately placed in the holder, the tail extended fully, and passed through a hole in a 2-inch lead shield to be attached next to an Eck and Krebs type Geiger counter tube. A continuous series of counts were taken until a sudden increase in the counting rate showed that radioactivity was present in the circulation of the rat's tail. The time was noted and the rat immediately killed, the abdomen opened, and the site of disintegration established by locating the gentian violet stain which showed where the coating had ruptured. The distance from the site of disintegration to the stomach was divided by the entire length of the small intestine. This decimal fraction of the length of the intestine was used to express the intestinal site of disintegration.

RESULTS OF *IN VIVO* TESTS

A high content of radiosodium gave a sharp indication of the time of break in the enteric coating. Figure 1 depicts a typical curve plotted with counts per minute against time, to show the sudden increase caused by the appearance of radiosodium in the circulation of the rat's tail.

In 25 tests, it was established that 22 coats disintegrated in the intestines, one coat was found broken in the stomach after thirty-five minutes, and the two remaining were found intact in the stomach when the rat was killed ten hours after administration of the pill.

[1] Radioactive sodium used in these experiments was obtained by allocation of the U. S. Atomic Energy Commission and from Dr. D. J. Tendam of the Physics Department, Purdue University.

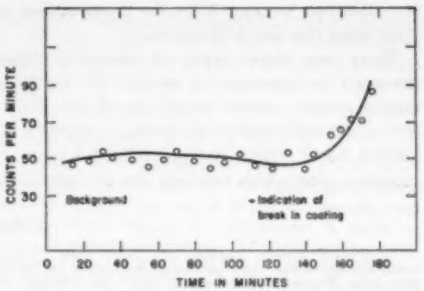

Fig. 1.—A typical curve: delayed appearance of radiosodium in the circulation.

The time for the appearance of radiosodium in the circulation consists of the time period the pill coating resisted gastric digestion and the time for intestinal disintegration. The total time in the intestines was calculated on the basis of two assumptions:

(*a*) The pill passed through the intestine at a uniform rate once it was emptied from the stomach.

(*b*) The pill which passed the farthest distance down the tract in the shortest length of time had passed out of the stomach without delay.

The value for the rate of travel for the pill, as determined by preliminary studies involving more than 75 tests (8), was found to be 0.009 of the total length of the intestine per minute. This rate of travel was divided by the decimal fraction of the total length to give the total time the pill was in the intestine. The time in the stomach and the intestinal disintegration time was calculated by difference.

Figure 2 shows the results of the *in vivo* tests and compares the intestinal disintegration time with time in the stomach. The individual tests arranged in order of time in the stomach show that there is no correlation between the time of disintegration and the time the pill remained in the stomach. This is to be expected if the enteric coating is truly "enteric," that is, acted upon slowly by the intestinal secretions to cause it to disintegrate. The pill is emptied from the stomach in a surge of liquefied food since the pylorus, as a rule, only allows liquids

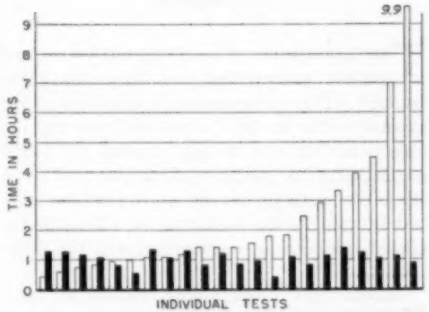

Fig. 2.—A comparison of: |——| , time in the stomach, and ▬, intestinal disintegration time, for individual tests.

to pass. In these tests 50% of the pills left the stomach within one and one-half hours, 75% within two and one-half hours, and 90% within four and one-half hours. There is no correlation between the time in the stomach and the amount of food in the stomach (8).

IN VITRO EXPERIMENTS

The apparatus used in these tests was that proposed by Thompson and Lee (9). The coated granules are placed in vials filled with artificial digestive fluid (10), and the vials fitted on a wheel in a spokelike manner. The wheel was suspended in a constant temperature water bath and rotated at about one revolution per minute. The pills moved from one end of the vial to the other as the wheel turned to simulate the movement of the gastrointestinal tract. The end point of the disintegration was recorded as the time for distinct coloring of the fluid by the amaranth in the sugar granules. These granules were given the enteric coat at the same time and under the same conditions as the radioactive pills.

The results of the *in vitro* tests show that the individual coating lots had similar disintegration characteristics. The average of the estimated intestinal disintegration time for the *in vivo* tests was sixty-one minutes which compares to sixty minutes average for the *in vitro* tests on all but one coating lot which was seventy-six minutes. This last lot also gave a gastric resistance time of eight hours, which compares to six and one-half hours average for the other lots. The *in vivo* test did not show any significant difference between the coating lots.

DISCUSSION

The advantages are that the devised method establishes the site and the time of disintegration. The remains of the pill can be studied to determine the manner in which the coating dissolved. These points are of interest in establishing the efficiency of a new enteric coating formula. The disadvantage of this method is that very small pills (or tablets) must be formed and coated. The coating should be relatively resistant to mechanical damage during the administration procedure.

The digestive system of the rat is comparable to that of man; however, the rat seems to have a milder acid reaction in the stomach than that of the average man (11) and also there is a continuous flow of the gastric juices and of the bile in the rat (12) which differs from the intermittent flow of these fluids in man (13).

SUMMARY

The radioactive tracer technique has been utilized to describe an *in vivo* method of testing enteric coatings.

(*a*) Radioactive sodium, as sodium chloride, made into small pills was coated and administered to a rat. The time and the site of disintegration was established by determining the time for the appearance of radioactivity in the circulation and by direct observation of the gastrointestinal tract.

(*b*) A method is described for estimating the time the coating resisted gastric digestion and the time for the coating to be dissolved in the intestinal fluid. These estimated periods are compared to a standard *in vitro* method.

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A Study of a Depressor Principle of *Veratrum viride**

By ARTHUR G. ZUPKO† and LEROY D. EDWARDS‡,§

A tentative extraction procedure for the isolation of a noncrystalline, long-acting depressor principle from *Veratrum viride* is presented. Studies on blood pressure, respiration, heart rate, and blood vessels in various species of animals indicate that the isolated depressor principle has an activity similar to that of an aqueous extract of the whole drug. A toxicological study including the LD_{50} in mice and rats and a histopathological study in rabbits and rats is reported.

THE dried rhizome and roots of *Veratrum viride* (green hellebore, American hellebore) have long been used as a cardiac depressant and hypotensive agent, especially in eclampsia and other conditions of high blood pressure. The injection of a physiological dose of an aqueous extract of *V. viride* is followed by a characteristic lowering of the blood pressure of long duration, transient slowing or stoppage of the respiration, and a slowing of the heart rate. Up to the present time all the principles responsible for these physiological actions have probably not been isolated, although much work has been done in this field since 1879 when Wright (1) reported the isolation of the alkaloids jervine, rubijervine, and pseudojervine.

Many workers have contributed to the present-day knowledge of the alkaloids and principles present in *V. viride*. Notable among these are Seiferle, Johns, and Richardson (2) who were the first to isolate germine and Jacobs and Craig (3, 4) who have isolated isorubijervine, veratrosine, veratramine, and a new unnamed alkaloid. These isolated constituents have been shown to be relatively inactive or comparatively weak in activity, especially with respect to vasodepression of long duration. However, recently (10, 11) the isolation of two long-acting esters of the alkamine, germine, from *V. viride* has been reported.

Previous workers have first isolated a principle, purified it, worked out its chemical configuration, and then tested it pharmacologically. In an effort to minimize the problem of working with relatively inactive principles, the present study was undertaken to first isolate a principle which would show activity similar to the aqueous extract of the whole drug and then to follow with further chemical study of that active principle.

EXPERIMENTAL

Isolation and Properties of the Depressor Principle

Extraction Procedure.—A variety of coarse and powdered samples of crude *Veratrum viride*, generously supplied by Irwin, Neisler and Company, Decatur, Ill., were used in this study. A majority of the samples contained 6% or more of acid-insoluble ash.

Extractions were done on a small scale, 1 to 3 Kg. of crude drug being used for each extraction. Approximately 100 extractions yielded an average of 25 mg. of the depressor principles per Kg. of crude drug. Fresh lots of the drug yielded up to 50 mg. of the depressor principle per Kg. and old samples yielded as little as 15 mg.

A total of five distinct residues was obtained from each sample and set aside for depressor activity tests. The extraction procedure is illustrated by the flowsheet.

Testing of the Residues.—Using the apparatus described by Williams, Harrison, and Grollman (5) for measuring systolic blood pressure in the tail of the intact unanesthetized rat, all five residues were given preliminary screening for vasodepressor activity. One group of 10 Wistar strain albino rats and a second group of 10 Wistar strain albino rats, made hypertensive by bilateral constriction of the renal artery, were used to test an individual residue.

Residues *A* and *B* (see flowsheet) were only weakly active, residue *C* was only slightly more active, and residue *Y* showed moderate vasodepressor activity. The *X* residue, henceforth called the depressor principle, exhibited consistent strong vasodepressor activity of long duration in both groups of rats. The depressor principle (*X* residue) was further tested on two cats and two rabbits and gave evidence of sufficient vasodepressor activity to warrant further investigation.

Properties of the Depressor Principle.—The chemical nature of the principle has not, as yet, been fully determined. On the basis of a heavy precipitate with Mayer's reagent and a positive nitrogen test the principle could be alkaloidal in nature. Aqueous solutions of the depressor principle respond basic to phenol red but not to phenolphthalein. Solubility of the principle in water suggests that it may also be glycosidal even though tests for sugar

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§ The authors are indebted to Dr. L. P. Doyle, Agriculture Experiment Station, Purdue University, for confirmation of pathologic findings.

Crude drug (1–3 Kg.)
Macerate with 95% ethyl alcohol (at least 3 times, 24 hr. each)
Filter (suction)
Concentrate to thick syrup (steam bath and reduced pressure)
Dry in air (remove all alcohol)
Take up in water (room temperature—not more than one liter)
Filter (suction)—removes resinous materials
Concentrate (steam bath and reduced pressure)
Wash several times with ether (removes aromatic substances)
Mix with fresh lead hydroxide and filter—removes organic acids, etc.
Make alkaline to pH 7.2 with sodium bicarbonate
Shake out with ether 5 to 6 times (30 min. for each)
Aqueous solution (II)
Ether solution (I)
Shake out with 2:1 CHCl3-Alc. (several times)
Evaporate to dryness
Take up in anhydrous ether, filter
Aqueous (A) (test)
CHCl3-Alc. (C)
Vacuum desiccate filtrate
Evaporate (test)
Take up in water at room temperature
Residue (Y)
Soluble part
Shake out with ether 5 to 6 times
Aqueous (B) (test)
Ether
Evaporate to dryness
Take up in ankydrous ether
Vacuum desiccate
(X) residue (test)

FLOWSHEET OF EXTRACTION

were essentially negative. Another possibility is that it is a mixture of two or more active depressor substances. The principle was also found to be soluble in alcohol, dilute mineral acids, chloroform, acetone, propylene glycol; slightly soluble in benzene and concentrated alkalies but insoluble in carbon disulfide.

An aqueous solution of 6 mg. % of the depressor principle was tested for polarographic activity using an 0.1 *M* solution of tetraethylammonium bromide as the positive electrolyte. The polarograph showed no reducible ions under the stipulated conditions and can therefore be considered as polarographically inactive.

A melting-point determination was done on the depressor principle extracted from each sample. A melting-point range of 93–108° (uncorrected) was obtained *via* the capillary method.

Preliminary attempts at crystallizing the depressor principle met with little success. The HCl salt obtained by precipitating from anhydrous ether with HCl gas was a fine amorphous precipitate which lowered blood pressure, but the duration of activity was considerably shorter than that of the crude depressor principle.

Upon long standing, aqueous solutions of the depressor principle lose much of their activity. Strong alkalies (6) and acids also diminish the activity, presumably due to a splitting-off of a chemical linkage necessary for prolonged vasodepression.

Pharmacology of the Depressor Principle

Effect on Blood Pressure and Respiration.—The outstanding effects of moderate doses of an aqueous extract of *Veratrum viride* injected intravenously in animals are a fall in blood pressure of long duration and a transient slowing or stoppage of respiration. These same physiological responses were observed with the depressor principle in experiments on dogs, cats, rabbits, rats, and roosters.

The duration of vasodepressor activity varied with the species of animal used, the rooster exhibiting only a comparatively transient fall in blood pressure lasting no more than fifteen minutes. *Via* intravenous injection, the depressor principle effectively lowered blood pressure for as much as five hours in

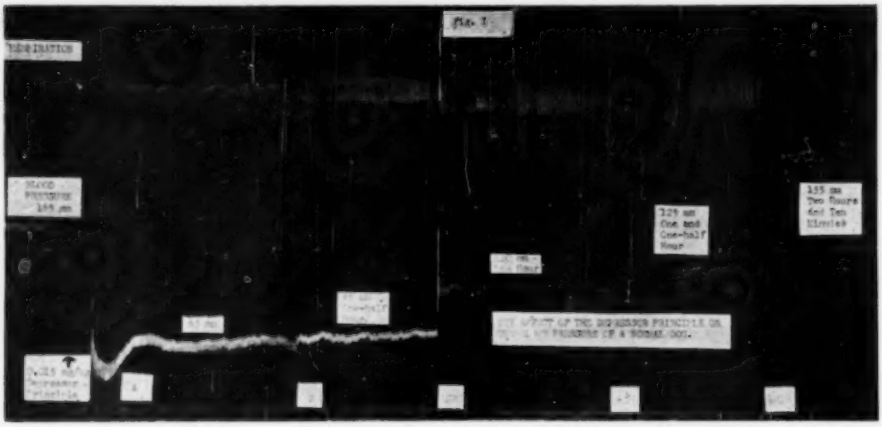

Fig. 1.—The effect of the depressor principle on the blood pressure of a normal dog. *A* shows the normal blood pressure and the result of an intravenous dose of 0.015 mg./Kg.; *B*, *C*, *D*, and *E* show the gradual rise in blood pressure over a two hour and ten minute period. Time interval = one minute.

the other species. When administered intramuscularly in dogs the blood pressure was lowered for almost seven hours without toxic manifestations.

A transient slowing of the respiration was observed in the dog, cat, and rabbit after intravenous injection of the depressor principle. However, the respiration was not slowed appreciably in dogs injected intramuscularly. Figures 1 and 2 show the effects of a small dose of the depressor principle when administered intravenously and intramuscularly, respectively.

Several experiments were conducted on cats in an effort to ascertain the role played by the autonomic nervous system in the mechanism of the vasodilator response exhibited by the depressor principle. It was found that the principle still acted after vagal paralysis with atropine, after paralysis of the sympathetic augmentory nerve fibers with ergotoxine, and after autonomic ganglia blockage with tetraethylammonium bromide. However, the duration of vasodepressor activity was diminished somewhat in each case, indicating that the autonomic system plays some role in the mechanism of action.

Effect on the Heart Rate.—Experiments on five intact dogs indicated that the heart rate is slowed significantly after an intravenous dose of 0.02 mg./Kg. or an intramuscular dose of 0.1 mg./Kg. of the depressor principle. With an intravenous dose the heart rate was lowered from an average of 152 beats

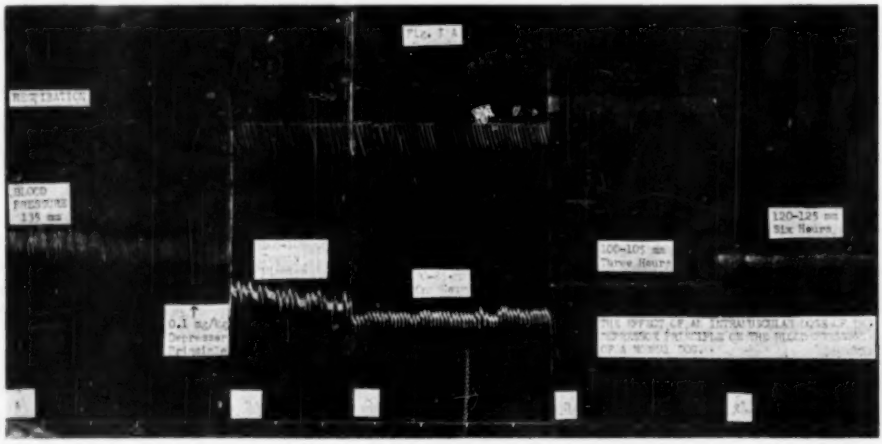

Fig. 2.—The effect of an intramuscular dose of the depressor principle on the blood pressure of a normal dog. *A* shows the normal blood pressure; *B* shows the gradual lowering twenty minutes later; *C* shows the maximum lowering reached over a one-hour period; *D* and *E* show the gradual rise in blood pressure three and six hours later, respectively. Time interval = one minute.

per minute to 97 beats within three minutes. At the end of an hour the heart rate was 124 beats per minute. With an intramuscular dose the heart rate was not slowed until fifteen minutes after injection. From a normal average heart rate of 151, the beat was slowed to an average of 135 at the end of a fifteen-minute period and 134 at the end of an hour.

Small doses of the depressor principle did not slow the isolated heart of pithed frogs and turtles. It is apparent that the central nervous system must be intact before a slowing of the heart rate is seen with the depressor principle.

When the isolated frog heart was made hypodynamic by perfusion with Hulse's perfusate (deficient in calcium ions), administration of the depressor principle increased the rate and contractile power of the failing heart.

Effect on the Blood Vessels.—The isolated hind legs of five rabbits were perfused according to the method described by Willson and Smith (7). The normal perfusion rate varied from 20 to 25 drops per minute. After a 0.3-cc. dose of 1:10,000 aqueous solution of the depressor principle, the perfusion rate showed some increase, but the number of drops never exceeded 49 per minute.

Using the Trendelenburg method, the hind legs of ten pithed frogs were perfused with the depressor principle. Little or no vasodilatation of the blood vessels was seen.

A group of three rabbits was used to determine the effect of the depressor principle on the coronary flow of the isolated heart using a modified Langendorff technique. The average volume of perfusate flowing through the coronaries over a two-minute period was 16.1 cc. in the normal heart. After injection of 0.1 mg. of an aqueous solution of the depressor principle the average volume of perfusate through the coronaries was 16.9 cc. over a two-minute interval. With a 0.3- and 0.5-mg. dose the volume was approximately 15.9 cc. These preliminary experiments indicate that the depressor principle has little or no vasodilator effect on the coronary vessels of the isolated rabbit heart.

The vasodilator action of the depressor principle cannot be explained by a direct influence on the vessel wall but is probably transmitted to the motor effectors *via* the nervous pathways.

Acute Toxicity.—The LD_{50} of the depressor principle was determined subcutaneously and intravenously in rats and subcutaneously in mice. Wistar strain albino rats weighing between 125 and 325 Gm., approximately 50% male and 50% female, maintained at a temperature of 22 ± 1° and fasted overnight, were used.

The LD_{50}, as calculated by the graphic method of DeBeer (8), for the depressor principle injected subcutaneously in rats was 1.59 ± S.E. 0.0102 mg./Kg. Table I shows the results of this determination. The LD_{50} for an intravenous injection in rats was 0.273 ± S. E. 0.0104 mg./Kg. The results are shown in Table II. In mice the LD_{50} of a subcutaneous dose was found to be 1.12 ± S.E. 0.10245 mg./Kg. These results are shown in Table III.

Chronic Toxicity.—Histopathological studies were done on a group of rats injected subcutaneously with the depressor principle daily for sixty days and a group of rabbits injected intravenously daily for thirty and forty-five days.

TABLE I.—RESULTS OF SUBCUTANEOUS INJECTIONS OF THE DEPRESSOR PRINCIPLE INTO WISTAR STRAIN ALBINO RATS

Group No.	Dose, Mg./Kg.	Mortality 24 Hr.	%
1	3.00	10/10	100.0
2	2.75	10/10	100.0
3	2.50	9/10	90.0
4	2.25	9/10	90.0
5	2.00	8/10	80.0
6	1.75	8/10	80.0
7	1.50	6/10	60.0
8	1.25	2/10	20.0
9	1.00	0/10	0.0
10	0.75	0/10	0.0

TABLE II.—RESULTS OF INTRAVENOUS INJECTIONS OF THE DEPRESSOR PRINCIPLE INTO WISTAR STRAIN ALBINO RATS

Group No.	Dose, Mg./Kg.	Mortality 24 Hr.	%
1	0.50	10/10	100.0
2	0.45	10/10	100.0
3	0.40	9/10	90.0
4	0.35	8/10	80.0
5	0.30	7/10	70.0
6	0.25	4/10	40.0
7	0.20	2/10	20.0
8	0.15	1/10	10.0
9	0.10	0/10	0.0
10	0.05	0/10	0.0

TABLE III.—RESULTS OF SUBCUTANEOUS INJECTIONS OF THE DEPRESSOR PRINCIPLE INTO SWISS STRAIN MICE

Group No.	Dose, Mg./Kg.	Mortality 24 Hr.	%
1	2.25	10/10	100.0
2	2.00	10/10	100.0
3	1.75	9/10	90.0
4	1.50	7/10	70.0
5	1.25	4/10	40.0
6	1.00	5/10	50.0
7	0.75	3/10	30.0
8	0.50	1/10	10.0
9	0.25	0/10	0.0

(*A*) A group of 17 Wistar strain albino rats, approximately 50% male and 50% female, weighing between 140 and 200 Gm., were housed in individual cages and maintained at a temperature of 22 ± 1°. The animals were placed on a routine rat pellet diet with no limitations on water consumption. Fifteen of these rats were given a daily subcutaneous dose of 0.4 mg./Kg. of the depressor principle, representing 25% of the determined median lethal dose. The other two rats, one of each sex, were used as controls and received no injections. The rats were weighed every three days to adjust for dosage levels. A total of 60 doses was administered at the end of which time the animals were sacrificed, autopsied, and any gross pathological changes noted. Five-millimeter sections of tissue were cut from the heart, liver, lungs, kidneys, stomach, spleen, and intestine of each animal and placed in Bouin's fixative solution overnight. The ensuing steps of dehydration, embedding in paraffin, blocking, cutting, staining, and mounting were all done according to the method

outlined by Zupko and Edwards (9). The prepared sections were then examined microscopically for any histopathological changes.

Thirteen of the fifteen rats receiving the depressor principle survived the sixty-day dosage period, one dying after 22 doses and the other after 37 doses. These early deaths were due to bacterial infection as evidenced by the extensive leucocytic infiltration seen in the lung sections of these animals. The surviving rats showed a weight increase as did the controls. The rats exhibited a characteristic syndrome after injection, viz., excessive salivation, some degree of depression, and continued retching for a five- to ten-minute period. Complete recovery from all symptoms ensued in about thirty minutes. There were no signs of irritation or abscess formation at the sites of injection.

There was no evidence of any gross pathology. No histopathological changes were seen microscopically in the heart, intestines, lungs, or stomach. The liver was normal except for a few cases where the nuclei did not stain well, but these areas were infrequently observed. The spleen showed a paucity of splenic pulp as seen in Fig. 3. The sinusoidal spaces and small blood vessels were also dilated. The germinal centers and the traveculae were normally spaced. The kidneys showed the greatest pathological change in the form of varying degrees of granular degeneration of the epithelium of the proximal convoluted tubules as illustrated in Fig. 4. An increased area of space between the tufts and Bowman's capsule was also observed in some cases. Occasionally a necrobiotic area was visible.

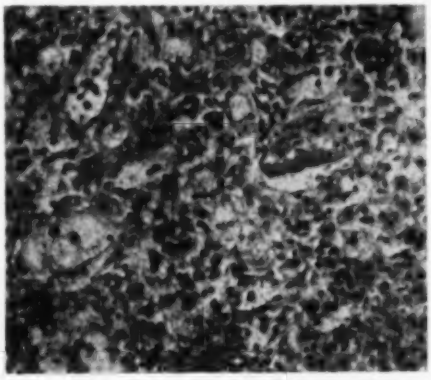

Fig. 3.—Rat spleen showing the paucity of splenic pulp and dilatation of the sinusoidal spaces and small blood vessels. (Magnification 210 ×.)

(*B*) A group of six male rabbits weighing between 2 and 3 Kg. was maintained under similar conditions as described for rats. The animals were given intravenous doses of the depressor principle (0.1 mg./Kg. *via* ear vein), one group of three rabbits being given 30 doses and the second group of three rabbits being given 45 doses. After the dosage periods the animals were sacrificed and tissue sections of the organs prepared in the same manner as described for rats.

Fig. 4.—Rat kidney showing granular degeneration of the epithelium of some tubular cells. (Magnification 210 ×.)

All the rabbits survived the dosage period. No salivation or slobbering was noted, but there was a small amount of depression and retching seen soon after administration of the depressor principle. All the rabbits showed a normal weight increase during the test period. There were no signs of irritation or abscess formation at the sites of injection.

No evidence of gross pathology was seen in either group. There were no histopathological changes seen microscopically in the heart, stomach, liver, lung, or intestine of either group. The spleen showed very little diminishment of splenic pulp, and only a few sinusoidal spaces and small blood vessels were greatly dilated. The kidneys showed a small amount of granular degeneration but not comparable to that seen in the rats. Only the epithelium of the proximal convoluted tubules showed any degree of consistent pathological change. Generally, only a small amount of pathology was noted in either group of rabbits, and the changes seen were almost solely confined to the kidneys.

SUMMARY AND CONCLUSIONS

1. A tentative extraction procedure for the isolation of a noncrystalline, ether-water soluble, long-acting depressor principle from *Veratrum viride* has been presented.

2. The ability of the depressor principle to lower blood pressure has been adequately demonstrated on five species of animals, viz., the dog, cat, rabbit, rat, and rooster.

3. Intramuscular injection of the depressor principle in dogs results in a gradual lowering of blood pressure, no respiratory embarrassment, and prolonged duration of activity due to slow absorption.

4. The heart rate was slowed with the depressor principle only when the central nervous system was intact, indicating a central or neurogenic action since the heart rate was not slowed in the isolated heart of pithed turtles and frogs.

5. Perfusion experiments on the hind leg of rabbits and frogs indicated little, if any, direct vasodilatation.

6. The LD_{50}, as calculated by the graphic method of DeBeer for the depressor principle injected subcutaneously in rats, was found to be 1.59 ± S.E. 0.0102 mg./Kg.; injected intravenously it was found to be 0.273 ± S.E. 0.0104 mg./Kg. In mice the subcutaneous LD_{50} was found to be 1.12 ± S.E. 0.10245 mg./Kg.

7. Histopathologic studies on rats and rabbits indicated there was little effect on the organs except for the kidney and spleen. The paucity of splenic pulp and the varying degrees of granular degeneration were the outstanding changes observed.

8. The mechanism of action of the vasodilator response exhibited by the depressor principle has not been fully clarified. It appears to be of a central or reflex neurogenic nature.

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A Study of the Seeds of *Strophanthus sarmentosus* and Some Related Species of *Strophanthus**

By HEBER W. YOUNGKEN and VARTKES H. SIMONIAN†

A report is given of the results of gross and histological studies of the seeds of *Strophanthus sarmentosus* P.DC. and related species, principally *S. gratus, S. Nicholsoni,* and *S. Courmonti.*

RECENT INTEREST in *Strophanthus sarmentosus* arose with the discovery of the latest miracle drug, Cortisone.

Although Cortisone cannot be isolated from the seeds of *S. sarmentosus*, Jacobs and Heidelberger (1) isolated sarmentogenin (from which Cortisone may be synthesized) from an alleged lot of *S. sarmentosus* seeds.

As is already apparent from recent publications, especially the excellent survey work in taxonomy by Monachino (2), the identity of the seeds which originally yielded sarmentogenin was not conclusively established to be *S. sarmentosus*, for subsequent samples of genuine *S. sarmentosus* seeds did not always yield sarmentogenin or yielded it in a lesser extent (1).

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† The authors gratefully acknowledge the donations of the various seeds used in this investigation by Professor T. E Wallis, Mr. Joseph Monachino, and Dr. J. C. Munch.

The confusion in the identities of various *Strophanthus* seeds is not new, for ever since *Strophanthus* was first suggested as having a cardiac action by the explorer David Livingstone (3), Holmes (4–6), Hartwich (7), Franchet (8), Jelliffe (9), Mathiesen (10), and Perredes (11) have attempted to classify and establish the possible types of *Strophanthus* seeds found in commerce.

The difficulties encountered in classifying the commercial seeds arise from the fact that such samples of *Strophanthus* seeds very frequently consist of mixtures of seeds derived from various species of *Strophanthus*.

It has been suggested that *Strophanthus* seeds be imported in their original follicles but such procedure is no guarantee of the authenticity of the seeds, since no dependable data exist to show how far the follicles vary in their morphology on plants of different age or when the same species grows under different ecological conditions.

While some of these *Strophanthus* seeds differ in their external characters, yet many others obtained from different species of *Strophanthus* present a marked similarity in shape, color, and size. Unfortunately, the morphological charac-

ters of these seeds vary to some extent in the same follicle, since the shape, size of the seed, and the length of its awn are dependent upon the position the seed occupies in the pod. The seeds in the middle of the pod are usually the most developed in respect to their awns while those nearest the base of the pod are often shorter and thicker.

It is, therefore, obvious that when seeds are removed from their pods it is not easy to find constant external morphological characters wherewith to separate or definitely identify seeds of different species of *Strophanthus*.

Some which appear identical to the naked eye may be derived from very different species and may differ very considerably in their histological features, in their cell contents, and their color reaction with 80 per cent sulfuric acid.

This study was undertaken in order to establish a criterion whereby a certain means of identification of the seeds of *S. sarmentosus* and related species of *Strophanthus* might be possible. The authors plan to extend their studies to seeds of additional species of *Strophanthus*.

The various authenticated samples of *S. sarmentosus* seeds discussed in this report were obtained as follows:

From Mr. J. Monachino of The New York Botanical Garden:

(*a*) *S. sarmentosus* seeds—"Callens."
(*b*) *S. sarmentosus* seeds—"Jacobs."

From Dr. J. C. Munch of Munch Research Laboratories:

(*c*) *S. sarmentosus* seeds from the lot used by Jacobs.

From Dr. T. E. Wallis of The Pharmaceutical Society of Great Britain, botanically verified seeds of:

(*d*) *S. sarmentosus.*
(*e*) *S. gratus.*
(*f*) *S. Courmonti.*
(*g*) *S. Nicholsoni.*

Having primarily examined all the aforementioned *S. sarmentosus* specimens, our conclusion is that the Jacobs and Munch samples were identical in structure and compared very closely with the Wallis sample although not identical with it in every detail. The Callens sample showed a similarity to those previously mentioned in respect to general tissue details but differed chiefly in respect to the form of the units of the crystal aggregates of calcium oxalate found in the cotyledons. The differences in the histology of these seeds are of such a minor nature as to warrant our belief that all of these four lots represent seeds of *S. sarmentosus* or forms or varieties of this species.

As a basis for authentic *S. sarmentosus* seeds we chose Father H. Callens' specimen, for this was supported by a herbarium specimen of the plant yielding the seeds which we obtained from The New York Botanical Garden (see Fig. 1) and which was botanically verified by Mr. Monachino.

MORPHOLOGICAL DESCRIPTION OF *STROPHANTHUS* SEEDS

***Strophanthus sarmentosus* (Fig. 2)**

Seeds ovate-lanceolate, reddish brown to brownish gray with a greenish to yellowish tinge, averagely 10 mm. in length, 4 mm. in breadth, and about 2 mm. in thickness, the apex with a marked twist, the base obtuse and somewhat flattened; external surface covered with thin-walled, unicellular hairs of variable length, the apices of which are not very sharp-pointed.

Histology.—When the seed was sectioned transversely at about its middle point between the base and the apex, the cross section was elliptical in outline with a slight ridge at each of the narrow lateral ends. The ridge was formed from the seed coat tissues alone. The epidermis of the spermoderm consists of a single row of large tabular cells with moderately thin tangential walls and thick anticlinal walls, the latter forming a spindle-shaped thickening where the adjacent epidermal cells meet. Numerous thin-walled nonglandular hairs of pale yellow color and up to about 230 μ in length arise as outgrowths of the outer walls of the epidermis. Many of these hairs are slightly wavy in aspect with soft pointed apices. The entire epidermal tissue including the hairs is only slightly lignified. When viewed under polarized light it exhibits a glistening aspect. In surface section, the epidermis exhibits elongated cells with irregularly wavy anticlinal walls, the latter being about 7.6 μ to 11 μ in diameter. The middle lamella appeared darker and more lignified than the rest of the walls. Beneath the epidermis is a more or less distinct hypodermis, some of the cells of which contain rhombohedral crystals or aggregate clusters of calcium oxalate. The crystal-bearing hypodermis forms the first layer of the pigment tissue which consists of from three to five rows of tangentially elongated parenchymatous cells of brownish aspect.

The endosperm consists of seven to nine rows of isodiametric cells with thick cell walls and containing aleurone grains and fixed oil globules. The endospermic tissue is of fairly uniform range of breadth in different sections, cut through the seed at many intervals, and surrounds two well-developed cotyledons. The mesophyll cells of the cotyledons contain numerous aleurone grains and oil globules. Each cotyledon possesses a conspicuous plerome strand extending through its center. Calcium oxalate crystals of the monoclinic prism type are numerous in the cotyledons and occur in clusters or conglomerates, rarely as single crystals.

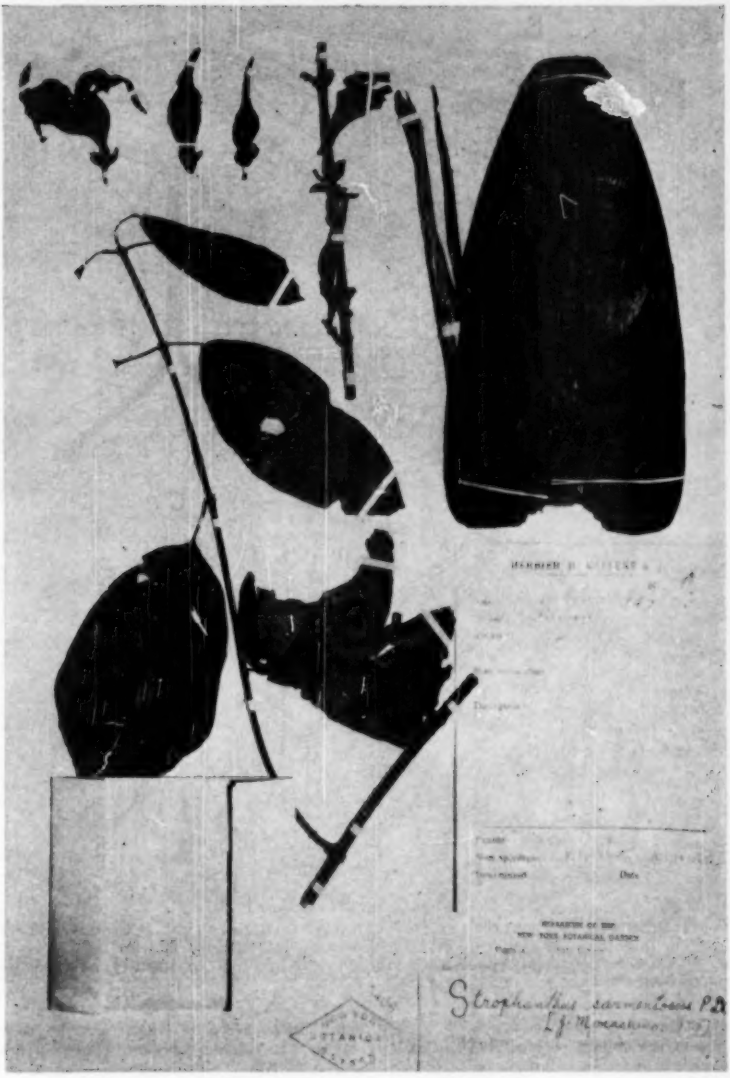

Fig. 1.—Photograph of herbarium sheet of *S. sarmentosus* P.DC. Collected by Fr. H. Callens in the Belgian Congo.

Transverse sections of the seed, mounted in 80% H_2SO_4, exhibit within a few seconds a pale rose-red color which develops first in the endosperm and then in the cotyledons. The most pronounced red color is in the plerome regions of the cotyledons.

Strophanthus Nicholsoni (White Woolly *Strophanthus*) (Fig. 3)

Seed broadly lanceolate, averagely about 15 mm. in length, about 5 mm. in breadth, and about 3 mm. in thickness, its surface densely covered with long white to pale yellow, glistening, hairs. The colors of the seed appear white when viewed at the base and yellowish-brown when viewed from the apex.

Histology.—The spermoderm is characterized by numerous irregular ridges projecting outward. Each ridge represents an outgrowth of epidermis and pigmented hypodermal parenchyma. Both the endosperm and mesophyll of the cotyledons possess isodiametric, thin-walled, reserve parenchyma cells with aleurone and fixed oil contents.

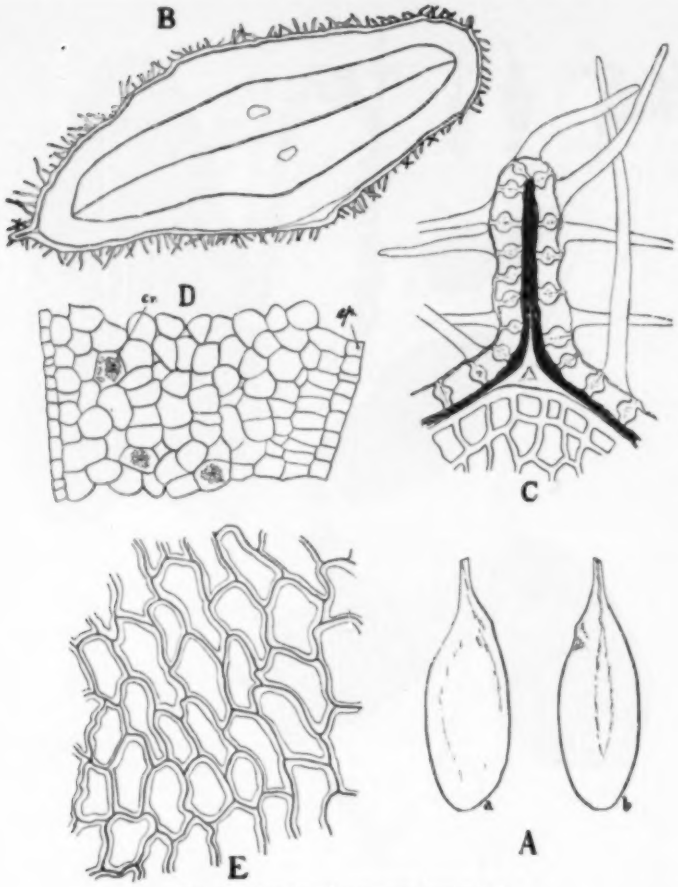

Fig. 2.—*Strophanthus sarmentosus* P.DC.

A, Views of the entire seed without the awn; (*a*) ventral and (*b*) dorsal views. *B*, Cross section of the entire seed at about the middle of the seed. *C*, T. S. of seed at the lateral ridge showing hairs, seed-coats, and endosperm. *D*, Cross section of cotyledon showing (cr.) crystals and (ep.) epidermis. *E*, Surface view of the spermoderm without the hairs.

Sections of the seed mounted in 80% H_2SO_4 exhibit a slowly developing play of colors starting with light yellow through yellowish, pale rose to red and finally violet.

***Strophanthus Courmonti* (Fig. 4 *A, B, C*)**

Seeds lanceolate to broadly lanceolate, mostly brown but sometimes brownish gray or greenish gray, averagely about 9 mm. in length, about 3 mm. in breadth and from 1 to 1.5 mm. in thickness; external surface covered with light yellow hairs but the hairs are not as dense as on seeds of *S. Nicholsoni*. The raphe is less marked than in *S. Kombé*.

Histology.—The spermoderm in cross section exhibits an epidermis of tabular cells whose radial walls are spindle-shaped. Numerous lignified, nonglandular hairs arise from the outer walls of the epidermal cells. In the sections examined they were up to 570 μ in length, and up to 18.5 μ in breadth in the basal region. The hair apices were soft pointed. The subepidermal region of the seed coat possesses numerous cubical crystals of calcium oxalate which occur in single prisms, twin prisms, clusters, and conglomerates. The endosperm is devoid of calcium oxalate crystals. The cotyledons are slightly unequal in size and in cross sections tapered to slender pointed ends. They contained no crystals.

Sections mounted in 80% H_2SO_4 slowly developed a yellow color, then orange red and, after ten minutes, a violet color.

***Strophanthus gratus* (Fig. 4 *D, E, F*)**

The seeds of this species, a source of ouabain, were long known under the scientific and commercial names of *"Strophanthus glaber"* until Franchet (8) determined the botanical origin to be *Roupellia*

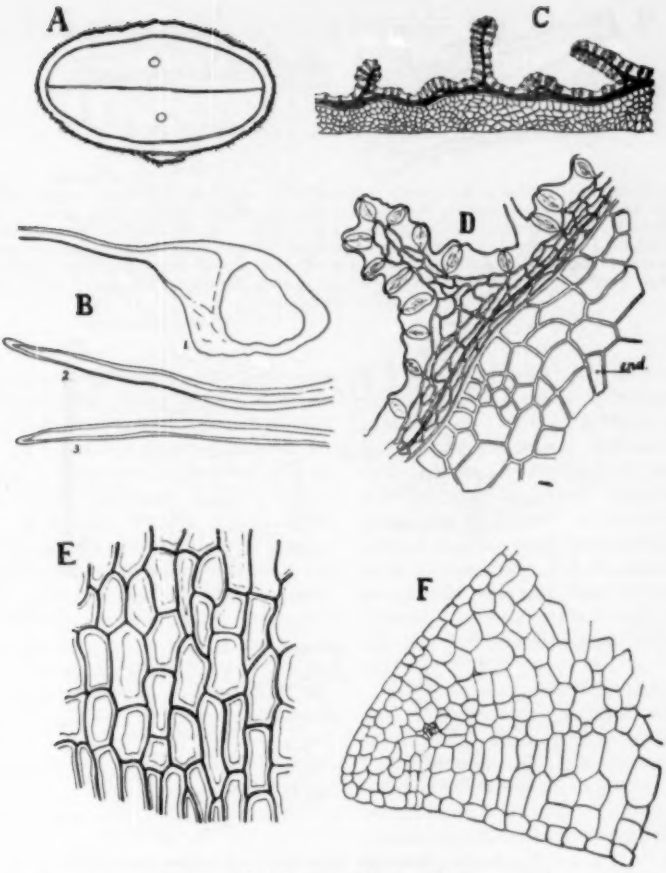

Fig. 3.—*Strophanthus Nicholsoni* Holmes.

A, Cross section of the entire seed at about the middle of the seed. *B*, Hairs; *1*, showing the base of a hair; *2* and *3* showing the ends. *C*, Cross section of seed to show the ridges which cover the entire seed. *D*, One of the ridges highly magnified to show seed-coats and endosperm. *E*, Surface view of the spermoderm without the hairs. *F*, Cross section of a cotyledon.

grata, which name was subsequently changed to *Strophanthus gratus* Baillon.

These seeds are glabrous, more or less fusiform, irregularly flattened and irregularly twisted with sharp angles along the margin, dull brown of uneven intensity on the surface, 15 to 19 mm. in length, about 2.5 to 3 mm. in breadth and 1 to 1.5 mm. in thickness. No hairs occur on the surface. The raphe is prominent.

Histology.—Transverse sections are somewhat elliptical with an irregularly undulate margin. The spermoderm is devoid of hair outgrowths. Its epidermal cells possess spherical to lenticular thickenings in their double anticlinal walls. The endosperm tissue occupies a zone of from 9 to 16 layers of cells and surrounds two slightly S-curved cotyledons. Crystals of calcium oxalate are entirely absent in seed coat, endosperm, and cotyledons.

When cross sections are mounted and examined in 80% sulfuric acid, an orange to pink color develops.

SUMMARY

1. A brief history of the seeds of *Strophanthus sarmentosus* and attempts to classify commercial *Strophanthus* seeds is given.

2. A report is given of the results of a study of the gross morphological and histological characteristics of seeds of *S. sarmentosus*, *S. Nicholsoni*, *S. Courmonti*, and *S. gratus*.

3. The seeds of *S. sarmentosus* obtained from a pod of a large liane growing in Kimasi Forest, Belgian Congo, and supported by a herbarium

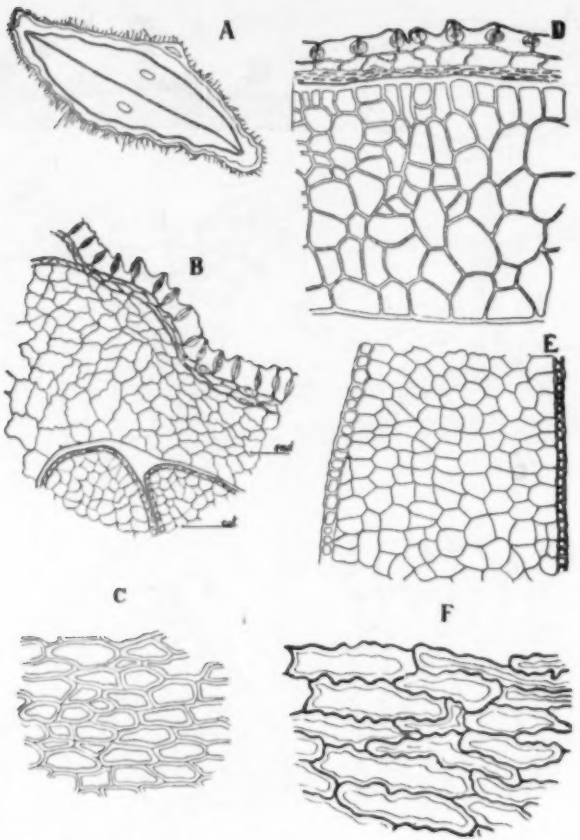

Fig. 4.—*Strophanthus Courmonti* Sacl. and *Strophanthus gratus* Baill.

A, Cross section of a *S. Courmonti* seed to show the entire outline. *B*, T. S. of *S. Courmonti* seed to show seed-coats, endosperm, and cotyledons. *C*, Surface view of *S. Courmonti* spermoderm. *D*, Cross section of *S. gratus* seed showing seed-coats and endosperm. *E*, Cross section of cotyledon in *S. gratus*. *F*, Surface view of *S. gratus* spermoderm.

sheet of this plant collected by Fr. H. Callens and botanically authenticated formed the basis for our description of the seed of *S. sarmentosus* in this paper. The Callens seed was compared with seeds from the lot used by Jacobs and Heidelberger in their isolation of sarmentogenin and with seeds botanically verified as *S. sarmentosus* by T. E. Wallis and found to be so similar in structure as to warrant our belief that all three lots of seed are those of *S. sarmentosus* or varieties or forms of that species.

4. The seeds obtained from J. C. Munch were structurally identical with the Jacobs and Heidelberger seeds.

5. The paper is illustrated by drawings illustrating the anatomy of the seeds of *S. sarmentosus*, *S. Nicholsoni*, *S. Courmonti*, and *S. gratus* and by a photograph of a herbarium sheet of a plant from which the Callens seeds of *S. sarmentosus* used in this investigation were obtained.

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The Influence of Tocopherols on U. S. P. XIV Vitamin A Assay*

By SERECK H. FOX and ALEXANDER MUELLER

The Morton-Stubbs procedure in the assay of vitamin A is subject to considerable over-correction when tocopherols are present. A procedure is described which circumvents the difficulty by employing a tocopherol-containing blank. The blank is prepared by shaking the vitamin A-tocopherol solution in hexane with 60 per cent sulfuric acid, followed by reading the acid-treated solution against the untreated solution. Data are presented indicating the effectiveness of the procedure in eliminating the difficulty.

IT HAS been observed that the analytical procedure for the estimation of vitamin A as prescribed in the United States Pharmacopeia XIV (1) results in low values when tocopherols are present in the sample in any significant amount. This is reflected in the Morton-Stubbs (2) type of correction, as specified by the Pharmacopeia. This over-correction becomes greater as the content of tocopherols is increased. Morton and Stubbs (2) have emphasized that their suggested correction procedure is based on the assumption that the irrelevant absorption values must be in linear arrangement over the range of the fixation points, and departures from linearity would produce an appreciable error in the corrected value for vitamin A.

The possibility of applying an additional correction factor to provide for the error introduced by tocopherols has been considered, but such a procedure has been found to be impracticable when the tocopherol concentration is unknown. Various attempts have been made to find a technique suitable for the separation of tocopherols from the vitamin A in the sample, but without success.

The possibility that the vitamin A might be destroyed without disturbance of the tocopherols was investigated and the procedure found to be practicable. This method is based upon the earlier procedure of Parker and McFarlane (3) in which 85 per cent sulfuric acid was employed for the purification of vitamin E-containing oils before estimation of vitamin E by colorimetric means.

Experiments employing sulfuric acid concentrations of the order of 85 per cent were found to give inconsistent recoveries of tocopherols. In the attempted construction of a blank for more satisfactory estimation of vitamin A in a vitamin A-tocopherol mixture, lower concentrations of acid improved the recovery. The data reported below were obtained by the use of sulfuric acid 60 per cent v/v. By carrying the vitamin A and tocopherol mixture into hexane solution it was possible to destroy the vitamin A with 60 per cent sulfuric acid without significant disturbance of the tocopherol. However, it was found to be important that the solutions be shaken exactly the same way each time, and also that contact of the tocopherols with acid must be restricted to the optimal period to prevent that destruction of tocopherol which will occur on undue exposure to acid.

Tocopherols are relatively unstable to boiling with alkali, as occurs in the saponification procedure. But, if pyrogallol is added as a protective agent (4) to the saponification mixture before the addition of alkali, reproducible tocopherol recoveries are obtained. Also more consistent vitamin A recoveries are obtained when pyrogallol is used.

EXPERIMENTAL

To illustrate the effect of tocopherols on the Morton-Stubbs correction, the spectral absorption curves, with and without added tocopherol, of a typical fish liver oil blend (unsap. fraction) are presented in Fig. 1. The curves with the legend at the bottom of the figure are largely self-explanatory. The following details, however, are pertinent. With no tocopherol present, the difference between the uncorrected value (*A*) and corrected value (*B*) is 16%. When 100 mg. of tocopherol is added to the original saponification mixture the difference amounts to 54%. The actual concentration of tocopherol present in the final reading solution is only 20 μg./cc. Of special significance is the calculated irrelevant absorption (after Morton-Stubbs) due to tocopherol (curve *F*), compared to the actual absorption contributed by the tocopherol (curve *G*). Due to the nature of the nonlinear irrelevant absorption given by tocopherol, the calculated irrelevant absorption is magnified ten times (i.e., from absorbancy of 0.025 to 0.265 at 325 mμ). This fact is also well illustrated by the data in Table I, which will be discussed later. The vitamin A oil used to obtain

* Received September 5, 1950, from R. P. Scherer Corp., Gelatin Products Div., Detroit, Mich.

TABLE I.—EFFECT OF TOCOPHEROLS ON U. S. P. VITAMIN A POTENCY WITH AND WITHOUT ACID BLANK

Number and Description	Theor. Potency U. S. P. Units	Unsap. Uncorr.	U. S. P. Potency	U. S. P. Potency Acid Blank
51059 Multivitamin Capsules	24,357	28,120	24,453	24,700
Above plus 50 mg. toco. VI[a]	24,357	28,500	21,447	23,807
Above plus 100 mg. toco. VI	24,357	28,800	18,787	23,408
Above plus 50 mg. toco. IV[b]	24,357	28,576	19,486	24,100
Above plus 100 mg. toco. IV	24,357	29,070	14,960	24,130
52327 HLO Capsules	4,662	5,491	4,959	4,940
		5,529	4,996	4,989
Above plus 15 mg. toco. VI	4,662	5,605	3,290	5,279
Above plus 30 mg. toco. VI	4,662	5,719	3,600	4,626
		5,747	3,517	4,863
Above plus 45 mg. toco. VI	4,662	5,672	3,515	4,617
53386 HLO Capsules	5,375	6,080	5,080	5,662
		6,137	5,337	5,595
Above plus 15 mg. toco VI	5,375	6,165	3,525	5,529
Above plus 30 mg. toco. VI	5,375	6,128	4,472	5,345
Above plus 50 mg. toco. IV	5,375	6,165	3,477	5,396
Above plus 60 mg. toco. VI	5,375	6,194	3,439	5,279
Above plus 75 mg. toco. VI	5,375	6,080	2,745	5,006
51425 Multivitamin Capsules containing 25 mg. toco. IV	26,780	29,640	24,600	27,100
52511 Multivitamin Capsules containing 25 mg. toco. IV	28,188	29,830	24,439	27,599
52512 Multivitamin Capsules containing 25 mg. toco. IV	28,188	29,452	23,465	27,835
52513 Multivitamin Capsules containing 25 mg. toco. IV	28,188	28,694	22,848	27,265
52760 Multivitamin Capsules containing 25 mg. toco. IV	25,680	30,162	23,798	25,792
52761 Multivitamin Capsules containing 25 mg. toco. IV	25,680	28,836	22,278	26,220
52762 Multivitamin Capsules containing 25 mg. toco. IV	25,680	30,635	23,418	28,120
53187 Multivitamin Capsules containing 25 mg. toco. IV	28,700	33,240	25,840	28,595
53188 Multivitamin Capsules containing 25 mg. toco. IV	28,700	32,577	24,937	29,022
52700 Multivitamin Capsules containing 7.4 mg. toco. VI	29,960	32,110	27,597	29,263
53213 Multivitamin Capsules with no toco.	12,000	13,495	10,616	11,614

[a] Natural Tocopheryl Acetate Conc. Type VI, Distillation Products.
[b] Natural Mixed Tocopherol Conc. Type IV, Distillation Products.

the data in Fig. 1 is the same oil used in a multivitamin mixture identified as No. 51059 in Table I.

Procedure.—The sample is prepared by saponification according to the U. S. P. XIV (1) with the exception that ca. 25 mg. pyrogallol (reagent grade) is added to the saponification flask before addition of the KOH. After saponification the unsaponifiable fraction is dissolved in sufficient hexane (spectrophotometric grade) to give an expected concentration of 8 to 15 U. S. P. units vitamin A per cc. Pipette 20 cc. of the hexane solution into a 30- to 35-cc. glass-stoppered reaction tube, and add 3 cc. 60% sulfuric acid (v/v). Stopper tightly and shake vigorously for fifteen seconds. Centrifuge immediately. Pipette 5 cc. of the acid-treated hexane solution into a volumetric flask and evaporate to dryness under inert gas (CO_2 was used) in a warm water bath. Cool and add exactly 5 cc. isopropanol. This solution is used for the blank to correct for tocopherol absorption. It is placed in the usual solvent or blank cell for reading against the tocopherol-containing vitamin A solution. The vitamin A solution is prepared from the original hexane solution by pipetting and evaporating a suitable aliquot and making to volume with isopropanol so that the concentration is 8 to 15 units per cc. The vitamin A dilution must be made on the same volume basis as the corresponding tocopherol blank dilution. Readings are taken in a Beckman Model DU Spectrophotometer according to U. S. P. XIV procedure. The slit width of the instrument is set against the tocopherol blank in the usual manner employed when only solvent is used in the blank cell.

Washing the hexane solution after centrifugation was found unnecessary. It is very important, however, that only dilute hexane solutions be used for the acid treatment. Stronger solutions in hexane, such as the original dilution after saponification, are likely to cause a temperature rise with the acid as well as poor separations on centrifuging, due to the larger residue.

DISCUSSION

A variety of pharmaceutical samples have been analyzed by this method for vitamin A in the presence of tocopherols. Table I shows examples of the effect of increasing concentrations of tocopherol on the calculated U. S. P. value with the vitamin A concentration being held constant and the improvement in results when a tocopherol blank is employed. Successive trials indicate a relatively good agree-

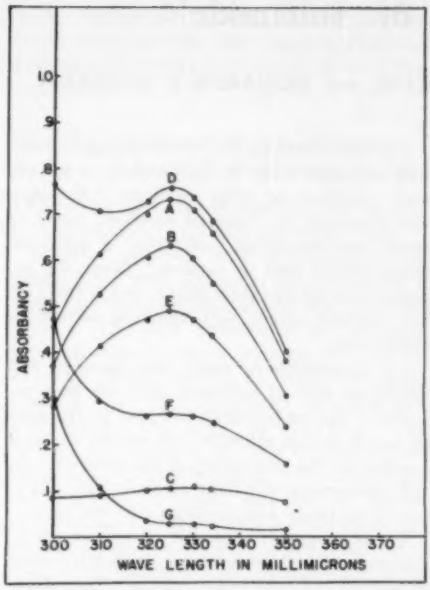

Fig. 1.—Spectral absorption curves of vitamin A in presence of tocopherol.

A. Vitamin A uncorrected.
B. Vitamin A Morton-Stubbs corrected.
C. Irrelevant absorption (by difference).
D. Vitamin A plus 100 mg. tocopherol VI, uncorrected.
E. Vitamin A plus 100 mg. tocopherol VI, Morton-Stubbs corrected.
F. Irrelevant absorption in presence of the tocopherol.
G. Absorption contributed by the 100 mg. tocopherol VI.

ment in spite of the difficulties which can occur. Some of the possible variables are the relative instability of both vitamin A and E on exposure to the lengthy preparation procedure, technique of the operators in carrying out the procedures, and most important the deviations which develop when small differences in absorbancy readings cause a considerable difference in final result when subjected to the calculation of corrected values. The results obtained by employing the tocopherol blank are less subject to variation than those obtained by application of the correction calculation. Work is in progress in this laboratory to collect data showing such differences. This will be reported at a later date.

The Type IV product, a concentrate of natural mixed tocopherols, causes a greater over-correction than the Type VI product (see Table I). Type VI consists chiefly of α-tocopherol in the form of α-tocopheryl acetate, while Type IV contains a higher percentage of non-α forms (in the free state), i.e., as tocopherols. The difference in applied correction is due to the difference in the location of their respective absorption peaks below 300 mμ. The absorption curve obtained when a vitamin A-tocopherol solution is read against the tocopherol-containing blank resembles the Morton-Stubbs corrected curve obtained (in the absence of tocopherol) by calculation. Results obtained on vitamin A samples with no tocopherol but employing the acid blank and without calculated correction also resemble the Morton-Stubbs calculated correction value. As might be expected, the 300/325 mμ and 350/325 mμ absorbancy ratios are considerably improved following the use of the acid-treated blank. The effect of sulfuric acid weaker than 60% has not been thoroughly investigated, but indications are that 60% is near the lower limit.

SUMMARY

The U.S.P. XIV analytical procedure for vitamin A employing the Morton-Stubbs correction gives low values when tocopherols are present. The low values are caused by nonlinear absorption contributed by the tocopherol. Spectral curves illustrating the interference are presented. Elimination of the difficulty by employing a tocopherol-containing blank, prepared by 60 per cent sulfuric acid treatment, improves the Morton-Stubbs correction. Results obtained after application of the acid-treated blank compare favorably with the theoretical values.

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Some Derivatives of Sulfamide*,†

By ED. F. DEGERING,‡ GLENN L. JENKINS, and BENJAMIN E. SANDERS§

The preparation and properties of compounds such as the carbomethoxy, carbethoxy, and dicarbalkoxy derivatives of sulfamide are described. The reaction of dimethylsulfamyl chloride with a number of amines was investigated and two new compounds are reported. A satisfactory procedure was developed for the preparation of ethyl pyrocarbonate. The pharmacological studies on these compounds will be reported later.

THE PURPOSE of this research was to synthesize derivatives of sulfamide which might have valuable pharmacological activity. There are few reports on their physiological action even though a considerable number have now been prepared. Aeschliman (1) reported that tetraethylsulfamide in small doses has a slight analeptic action. It was not found to have any antithyroid activity (2). Tzanck and co-workers (3) found *p*-aminophenylsulfamidopyridine to be too toxic for any medicinal use.

The N-substituted sulfamides and their methods of preparation have been reviewed by Audrieth and co-workers (4). Since that time a considerable number of acyl, tri- and tetraalkyl and aryl, heterocyclo- and cyclo-derivatives have been reported (5–9).

Some of these compounds have been tested and show considerable physiological activity but they have not been reported in the literature.

The synthesis of N-substituted sulfamides has been attained through the action of primary aromatic amines or primary and secondary aliphatic amines upon sulfuryl chloride, or by the action of an amine upon an (aryl)-arylsulfonyl sulfamyl chloride, an (acyl) arylsulfamyl chloride, a dialkylsulfonyl chloride, phthalylsulfamyl chloride, or phenyl chlorosulfonate (4).

The principal objective of this research was the preparation and study of carbalkoxy and other derivatives of sulfamide. Literally thousands of references are available on the activity of the carbamates as hypnotics, sedatives, local anesthetics, and a variety of other actions when other functional groups are present.

Chloroformates are not active enough to react with sulfamide even in the presence of sulfuric acid, pyridine, or other catalysts. Anderson and Degering (5) reported excellent yields of mono- and diacylated derivatives of sulfamide using sulfuric acid as catalyst. Glenn (9) reported that ketones react readily and O'Dell (10) had excellent success with aldehyde derivatives of sulfamide.

The preparation of mono- and disodium and potassium salts of sulfamide (11) has been reported. The monosodium salt may be prepared by use of sodium ethoxide, but sodium amide is required for the preparation of the disodium salt, and unless excess of sodium amide is used, a mixture of the mono- and disodium salts will result.

The heterogeneous reaction of sodium salt of sulfamide with chloroformates yields the desired carbalkoxy sulfamide in poor yields. The sodium salt is very hygroscopic and necessitates extreme care in handling. Numerous solvents were tried but none give very satisfactory yields.

The reaction of sulfuryl chloride with urethan is reported to give ethylallophanate (12). This evidently results from the formation of cyanic acid which immediately reacts with unchanged urethan to give the product described.

Sulfuryl chloride forms an addition compound with pyridine (13). This addition product is exceedingly unstable to heat. It was proposed that this sulfuryl chloride pyridine addition compound would gradually liberate sulfuryl chloride to react with the carbamate. As the solution was allowed to warm up slowly to room temperature excellent yields were obtained in the case of the unsubstituted carbamate. In the case of N-alkylcarbamates, the original carbamate was recovered largely unchanged, under conditions employed.

A number of reactions of dimethylsulfamyl chloride with ethyleneimines, N,N-diethylalkylene diamines, and ethylcarbamate gave products that could not be identified. However, the reaction of dimethylsulfamyl chloride with ethylenediamine, and with ethyl N-(2-aminoethyl)-carbamate gave products which could be isolated and identified.

Ethyl pyrocarbonate first described by Boehm and Mehta (14) was investigated. Since it is such an extremely reactive compound, it was thought that it might react directly with sulfa-

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mide. Boehm and Mehta prepared the compound from emetine, ethyl chloroformate, and potassium hydroxide, and postulated the following reaction:

$$2R_3\overset{+}{N}(\overset{-}{Cl})COOC_2H_5 + KOH \rightarrow R_3\overset{+}{N}H(\overset{-}{Cl}) + H_5C_2{-}OC(O)O{-}C(O){-}OC_2H_5 + KCl$$

Their yields with emetine were about 60 per cent but the reaction is expensive and impractical.

It was proposed that ethylchloroformate should react with potassium ethylcarbonate to give the ethylpyrocarbonate in any quantities desired. The best preparation of potassium ethylcarbonate for this experiment was found to be made by the addition of CO_2 to potassium ethoxide (15). The potassium ethylcarbonate then reacted with ethyl chloroformate. This reaction was found to be very satisfactory and simple; thus, a new preparation of a most valuable reagent. It reacts with amines, aminoalcohols, and a variety of other compounds that have not been thoroughly investigated. It is much more reactive than diethyl carbonate, or the chloroformates. There is no reason why a series of these pyrocarbonic esters could not be prepared in the same way.

EXPERIMENTAL

Preparation of Carbomethoxysulfamide.—A sample of 4.4 Gm. (0.045 mole) of dry sulfamide is dissolved in 100 ml. of absolute alcohol (distilled from magnesium methylate) in a flask fitted with a reflux condenser. To this flask is added 1.8 Gm. (0.05 mole) of metallic sodium. The monosodium salt of sulfamide precipitates during the refluxing of the alcoholic solution. This salt is then filtered with suction and heated over a steam plate until the odor of alcohol no longer can be detected. This is then added from a solid-addition flask to 50 ml. of redistilled methyl chloroformate (Eastman Kodak Co.) in a 200-ml. three-necked flask. It is added over a period of about three hours with stirring, and the temperature is maintained at that of refluxing chloroformate. It is heated for an additional two hours after all the sodium salt of sulfamide is added. It is allowed to stand overnight and 1.4 Gm. of crystalline substance settled out. There are several grams of the amorphous sodium salt of sulfamide. The crystalline material is washed with petroleum ether and isopropyl ether. It is recrystallized from ethyl acetate and the melting point is 124–125°. The yield on the basis of sulfamide used was about 23%.

Preparation of the Sodium Salt of Sulfamide and Its Reaction with Ethyl Chloroformate.—Commercial 200-proof ethanol is dried by means of magnesium methylate and later distilled. Four grams of sulfamide (0.041 mole) is added to 100 ml. of the hot alcohol. One gram (0.043 mole) of metallic sodium is added through the condenser. The sodium salt precipitates almost immediately. The solution is filtered with suction and the white powder dried in a vacuum desiccator.

Fifty milliliters of ethyl chloroformate is added to a 200-ml., three-necked, round-bottomed flask fitted with a stirrer, a condenser, and a solid-addition flask. The sodium salt of sulfamide is added from this addition flask to the refluxing ethyl chloroformate over a period of four hours. About 1 Gm. of white product is obtained and a dark yellow product is obtained in larger amounts. The dark yellow product appears to be a sodium salt, which is insoluble in alcohol and ether. The light crystalline material is very soluble in alcohol, water, and slightly soluble in ether. It is dissolved in ether, then later recrystallized from chloroform. The carbethoxysulfamide melts at 134–135°. This represents only about 16% yield.

Anal. Calcd. for $C_3H_6N_2O_4S$: N, 16.67. Found: N, 17.13.

Preparation of Carbethoxysulfamide in Dilute Alkali.—Sulfamide, 5 Gm. (0.052 mole) is added to 3% NaOH in a 200-ml. round-bottomed flask, fitted with a utility head (condenser, stirrer, and dropping funnel, all connected to one joint). Within two hours, 4.9 ml. (0.052 mole) of ethyl chloroformate is added to the cold alkaline solution of sulfamide. After the addition the flask is allowed to warm up to room temperature and stirring is continued for several hours. Removal of water is then accomplished by use of an aspirator. The white solid remaining in the flask is dissolved in ethyl ether. That which remains undissolved proves to be mainly sodium chloride. It is recrystallized from ethanol and melted at 132–134°. This method was not found to give good results consistently. An explanation probably can be found in the instability of sulfamide in alkali and the reaction of the chloroformate with the alkaline solution.

Preparation of N,N′-Dicarbethoxysulfamide from Sulfuryl Chloride, Urethane, and Pyridine.—To a 1-liter, three-necked, round-bottomed flask, fitted with a stirrer, condenser, and dropping funnel, is added 500 ml. of benzene (dried over sodium and distilled). To the benzene in the flask, 15 Gm. (0.169 mole) of urethan (recrystallized from benzene and dried in a vacuum desiccator) and 3.4 Gm. (0.169 mole) of pyridine is added. Over a period of three hours, 11.4 Gm. (0.085 mole) of sulfuryl chloride is added, while the temperature is kept around 0°. The flask is allowed to warm up to 30° and the solution is stirred vigorously for fourteen hours. From the benzene solution 36.1 Gm. of white, amorphous solid is obtained. It is washed several times with 10-ml. portions of dilute hydrochloric acid and filtered. The precipitate is washed with cold water to remove any hydrochloric acid remaining. It is extremely difficult to get rid of the last traces of pyridine. The precipitate is recrystallized from 95% ethanol forming rather thick, glassy crystals. It is recrystallized from alcohol five times, placed in an Abderhalden pistol, and dried at 10 mm. pressure for several hours, at the temperature of refluxing toluene. The melting point is 163–164° (uncorrected). A yield of 90% was obtained.

Anal. Calcd. for $C_8H_{12}N_2O_6S$: C, 30.1; H, 5.0; N, 11.7. Found: C, 30.1, 30.1; N, 5.2, 5.2; N, 11.62.

Preparation of N,N'-Dicarbopropoxy Sulfamide.—There is added to 400 ml. of anhydrous ether, 20.6 Gm. (0.2 mole) of propylcarbamate, and redistilled, dried, pyridine (0.2 mole) in a three-necked flask. Sulfuryl chloride (0.1 mole) previously distilled and diluted with 100 ml. of ether is added over a three-hour period to the solution. During this time the solution is cooled to 0° and stirred vigorously. The stirring is continued for an additional twenty-four hours while allowing the solution to rise to 30°. Fifty grams of crude product is obtained. After washing with dilute hydrochloric acid, 25 Gm. of comparatively pure product is obtained, which is about 94% of theoretical yield. The compound is found to be very slightly soluble in water, insoluble in benzene and ether. It was recrystallized from dilute alcohol and melted at 135°.

Anal. Calcd. for $C_8H_{16}N_2O_6S$: C, 35.82; N, 10.45. Found: C, 35.38, 33.55; N, 10.74, 10.92.

Preparation of 1,2-Bis-(N,N-dimethylsulfamylamino)ethane.—Ethylene diamine is rectified from concentrated sodium hydroxide solution. This ethylene diamine (7.4 Gm. or 0.11 mole) is added to 16 Gm. of dimethylsulfamyl chloride in a 1-liter flask containing 300 ml. of benzene. The solution is very vigorously stirred while being cooled with ice water. After all the dimethylsulfamyl chloride is added, the solution is heated for several hours. On filtration, about 10 Gm. of a light yellow solid is obtained. After removing the benzene, approximately 3 Gm. of white solid is recovered. This last solid is found to be soluble in methanol and ethanol, slightly soluble in isopropyl alcohol, and insoluble in petroleum ether. It is recrystallized from isopropyl alcohol several times and submitted for analysis after drying in an Abderhalden pistol. The melting point is 85° (uncorrected). The remainder of the solid which constituted the major portion of the yield gave a halogen test and has not been identified.

Anal. Calcd. for $C_6H_{12}H_4O_4S$: N, 20.44. Found: N, 20.38, 20.35.

Preparation of N-Carbethoxy-N'-dimethylsulfamylethylenediamine.—

$$Me_2N\cdot SO_2\cdot Cl + H_2N\cdot CH_2CH_2\cdot NH\cdot C(O)OEt \rightarrow Me_2N\cdot SO_2\cdot NH\cdot CH_2CH_2\cdot NH\cdot C(O)OEt + H_2NCH_2CH_2NH_2(2HCl)$$

Preparation of ethyl N-(2-aminoethyl)carbamate is accomplished from diethylcarbonate and ethylenediamine.

To 400 ml. of redistilled benzene is added 44 Gm. (0.33 mole) of ethyl N-(2-aminoethyl)carbamate. Then there is added to this solution, with constant stirring, 23.8 Gm. of dimethylsulfamyl chloride (0.16 mole). The solution is cooled during the addition period. Stirring is continued for six hours. The solution is then filtered and 24 Gm. of white solid is collected, which is nearly the theoretical amount of the hydrochloride of the original carbamate. The solvent is removed under reduced pressure. The residue, a rather viscous oil, is subjected to distillation at 0.005 mm. pressure. The Hickman still is heated with an oil bath, and the bath is gradually raised to 100°. A solid separates out on the top and sides of the still. Hot oil is placed on top of the still in order for the distillation to continue. The liquid collected is redistilled several times at this low pressure.

On the basis of the amount of the hydrochloride of the carbamate recovered, the reaction is practically quantitative.

Anal. Calcd. for $C_7H_{17}N_3O_4S_1$: N, 17.57. Found: N, 17.42.

Preparation of Pyrocarbonic Ester.—

$$KO\cdot Et + CO_2 \rightarrow KO\cdot C(O)OEt$$

then

$$KO\cdot C(O)OEt + Cl\cdot C(O)OEt \rightarrow EtO\cdot(O)C\cdot O\cdot C(O)OEt + KCl$$

Two moles of potassium was added to 400 ml. of redistilled toluene in a 1-liter, three-necked flask. The toluene was heated up to reflux temperature while stirring the solution actively. From a dropping funnel, 92 Gm. (2 moles) of absolute alcohol, previously distilled from calcium hydride, was added slowly. After addition of all the alcohol, the solution was cooled. Then carbon dioxide was passed through a drying train from a cylinder until the flask no longer heats up. A good stirrer, preferably paddle type, is necessary to keep this slurry moving. It is often necessary to add more toluene in order to keep the solution fluid.

Then two moles of ethyl chloroformate (217 Gm.) is added from a dropping funnel during a six-hour period. The solution is stirred for an additional three hours. At the end of this time 200 ml. of toluene is added to extract most of pyrocarbonic ester. Then cold water is added to the flask, and this mixture is shaken in a separatory funnel to remove salts. The toluene extracts and water-insoluble portions are combined and distilled with a vacuum pump. Toluene, ethyl chloroformate, and ethyl carbonate are removed below 40° at 12 mm. pressure. Pyrocarbonic ester is collected from 74–76° at 7 mm. pressure. Careful attention must be paid to heating pyrocarbonic ester for it is easily decomposed around 100°, especially if acid or base is present (yield: 50% of theoretical). A derivative is prepared from hydrazine hydrate, which melts at 132°. It reacts with almost explosive violence to form the symmetrically substituted compound.

SUMMARY

1. The preparation of carbomethoxy and carbethoxy sulfamide was accomplished.

2. A general method for the preparation of dicarbalkoxy derivatives of sulfamide was worked out.

3. The reaction of dimethylsulfamyl chloride with a number of amines was investigated and two new compounds were prepared.

4. An excellent method for the preparation of ethyl pyrocarbonate was found. This reagent was investigated and found to be extremely reactive with amines and alcohols.

5. Pharmacological testing of the compounds prepared will be carried out at a later date.

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The Manufacture, Solubility, and Stability of Hypodermic Tablets Containing Morphine Salts*

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Hypodermic tablets containing morphine sulfate, morphine and atropine sulfates, and the Schlesinger formula have been studied. The tablet bases used included lactose, lactose with ascorbic acid, lactose with sodium metabisulfite, and mannitol. The usual moistening agents were employed. The finished tablets were stored under accelerated aging conditions and then subjected to macroscopical examinations, rate of solubility tests, and quantitative alkaloidal assays. Using the recommended method of manufacture and storage, the finished products did not discolor, did not show any significant loss in alkaloidal content, and dissolved in water in a reasonable period of time, after storage for nearly a year under various accelerated aging conditions.

DISCOLORATION and slow solubility of hand-molded hypodermic tablets containing morphine salts have long presented a problem to the pharmacist. Since the inception of the manufacture of tablet triturates in 1861 by Fuller (1), studies have been made of various phases dealing with this dosage form. Kebler (3) conducted a study of the evolution of the tablet and gave a thorough review. Bower (4, 5) reported the factors causing variations in tablet yield. The work of Smithers (6) presented the effect of alcohol strength of the molding solution. The texts (7–9) contained the methods of manufacture in a general manner. Notwithstanding these investigations, the method remained relatively unchanged. Although this is a "slow method" (2), it has continued to be used, with some modification, by the professional pharmacist and by manufacturers.

EXPERIMENTAL

To learn if the discoloration and slow solubility were a result of hydrolysis and/or oxidation of the ingredients of the tablet, we selected diluents, antioxidants, solutions, and storage conditions which would modify these chemical and physical properties.

The control tablets contained the usual diluent, lactose. The modified diluents were lactose with 0.1% ascorbic acid, lactose with 0.1% sodium metabisulfite and the sugar alcohol, mannitol. The molding solution contained alcohol 30% (*v/v*), sucrose 5% (*w/v*), and distilled water. The water and sucrose in solution acted as binding agents, and the alcohol hastened the drying. Another solution, the moistening solution, used to moisten mixtures which had dried, contained alcohol 30% (*v/v*) in distilled water.

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The equipment used for the manufacture of the tablets consisted of a mold of hard rubber. The mold consisted of two plates. One plate contained 100 perforations. The other plate was fitted with a corresponding number of projecting pegs.

Hypodermic tablets of morphine sulfate, morphine and atropine sulfates, and the Schlesinger formula were the three combinations specifically studied. To determine the components responsible for the discoloration of the latter tablet, we prepared tablets of each ingredient and combinations of the various ingredients.

The materials were mixed by the geometric dilution method, triturated in a mortar, and sieved five times through a No. 80 sieve. A 0.5-cc. portion of the molding solution was added to each 4 Gm. of the mixture to make approximately 100 tablets. Occasionally it was necessary to add 0.1 to 0.2 cc. of the moistening solution to complete each molding. The finished tablets were dried at 50° for twenty-four hours, placed in a desiccator for forty-eight hours and assayed. Subsequent assays were run on tablets after storage at room temperature (22–34°) and at 50°, both at atmospheric conditions and in a desiccated atmosphere. The tablets stored at room temperature were assayed every three months; those at elevated temperature after one month. The finished tablets were subjected to macroscopical examination and to rate of solubility tests as well as to the alkaloidal assays.

TABLE I.—HYPODERMIC TABLETS OF MORPHINE SULFATE

Batch Number:	5		6		7		8	
Diluent:	Lactose		Lactose with Ascorbic Acid		Lactose with $Na_2S_2O_5$		Mannitol	
	A[a]	B	A	B	A	B	A	B
	Rate of Solubility (in Seconds)							
Initial	7.5	...	22.5	...	18.0	...	10.5	...
Three months	6.0	6.0	60.0	60.0	45.0	45.0	12.0	6.5
Six months	12.0	6.0	37.5	30.0	60.0	60.0	27.0	4.5
Nine months	6.0	6.0	45.0	51.0	60.0	54.0	15.0	9.0
	Appearance[b] (Color)							
Initial	W	W	W	W	W	W	W	W
Three months	W	+	W	++	W	W	W	W
Six months	W	+	W	++	W	W	W	W
Nine months	W	+	W	++	W	W	W	W
	Assay (Percentage)[c]							
Initial	50.04	...	50.41	...	50.09	...	51.63	...
Three months	49.10	48.44	47.46	46.57	51.87	50.49	48.94	46.65
Six months	47.47	46.61	46.65	45.61	48.36	48.02	48.92	46.74
Nine months	46.19	46.49	47.06	46.98	46.27	48.20	48.08	49.06
Nine months normal plus one month accelerated aging	44.67	45.95	46.25	45.40	44.21	43.00	46.75	47.72

[a] Room temperature storage: A = in desiccator; B = room atmosphere.
[b] Appearance: W = white, impart no color to solution; + = slightly discolored, impart pale straw color to solution; ++ = discolored, impart straw color to solution.
[c] 49.62% theoretical value.

TABLE II.—HYPODERMIC TABLETS OF MORPHINE AND ATROPINE SULFATES

Batch Number:	9		10		11		12	
Diluent:	Lactose		Lactose with Ascorbic Acid		Lactose with $Na_2S_2O_5$		Mannitol	
	A[a]	B	A	B	A	B	A	B
	Rate of Solubility (in Seconds)							
Initial	7.5	...	21.0	...	7.5	...	18.0	...
Three months	9.0	4.5	9.0	12.0	9.0	6.0	10.5	21
Six months	9.0	6.0	10.5	9.0	15.0	9.0	15.0	30
Nine months	4.5	15.0	24.0	9.0	13.5	12.0	15.0	15
	Appearance[b] (Color)							
Initial	W	W	W	W	W	W	W	W
Three months	W	+	W	+++	W	W	W	W
Six months	W	+	+	+++	W	W	W	W
Nine months	W	+	+	+++	W	W	W	W
	Assay (Percentage)[c]							
Initial	49.58	...	47.41	...	50.62	...	50.47	...
Three months	51.01	49.17	51.89	50.14	52.03	50.55	53.73	51.97
Six months	45.64	45.30	47.85	46.93	46.92	46.50	46.41	46.56
Nine months	45.35	45.58	46.49	47.01	47.06	45.64	46.89	45.99

[a] Room temperature storage: A = in desiccator; B = room atmosphere.
[b] Appearance: W = white, impart no color to solution; + = slightly discolored, impart pale straw color to solution; +++ = grossly discolored, impart yellow color to solution.
[c] 49.62% theoretical value.

TABLE III.—HYPODERMIC TABLETS OF SCHLESINGER FORMULA

Batch Number:	13		14		15		16	
Diluent:	Lactose		Lactose with Ascorbic Acid		Lactose with $Na_2S_2O_5$		Mannitol	
	A[a]	B	A	B	A	B	A	B
	Rate of Solubility (in Seconds)							
Initial	16.5	...	34.0	...	13.5	...	7.5	...
Three months	7.5	9.0	7.5	18.0	6.0	9.0	4.5	7.5
Six months	15.0	15.0	18.0	30.0	10.5	9.0	7.5	7.5
Nine months	12.0	30.0	27.0	45.0	7.5	15.0	6.0	12.0
	Appearance[b] (in Color)							
Initial	+	+	+	+	W	W	W	W
Three months	+	+	+	++	W	W	W	W
Six months	+	+	+	++	W	W	W	W
Nine months	+	+	+	++	W	W	W	W
	Assay (Percentage)[c]							
Initial	36.10	...	37.05	...	36.86	...	36.67	...
Three months	37.22	36.69	36.49	35.72	37.39	37.55	38.45	37.90
Six months	35.92	34.98	35.63	35.51	35.83	34.97	36.34	35.95
Nine months	35.34	34.61	35.82	36.26	36.00	36.26	36.09	35.72

[a] Room temperature storage: A = desiccator; B = room atmosphere.
[b] Appearance: W = white, impart no color to solution; + = slightly discolored, impart pale straw color to solution; ++ = discolored, impart straw color to solution.
[c] 37.15% theoretical value.

TABLE IV.—HYPODERMIC TABLETS, SCHLESINGER VARIATIONS, LACTOSE BASE

Batch No.	Storage at 50°:	Initial		One Month		Two Months		Theoretical Value
		A[a]	B	A	B	A	B	
17[b]	Appearance[c]	++	++	++	++	++	++	
	Assay	47.53	..	44.26	45.11	47.79	46.48	49.62
18	Appearance	W	W	W	W	W	W	
	Assay	47.64	..	48.41	47.68	49.12	48.23	49.62
19	Appearance	W	W	W	W	W	W	
	Assay	2.02	..	2.17	2.17	XXX	XXX	2.00
20	Appearance	+	..	++	++	++	++	
	Assay	33.54	..	35.22	34.90	35.21	35.29	37.15
21	Appearance	+	..	++	++	++	++	
	Assay	45.29	..	46.83	45.58	47.00	47.58	49.62
22	Appearance	W	W	W	W	W	W	
	Assay	48.65	..	48.74	49.20	49.08	45.11	49.62

[a] Room temperature storage: A = in desiccator; B = room atmosphere.
[b] 17 = H. T. Morphine HCl; 18 = H. T. Ethylmorphine HCl; 19 = H. T. Scopolamine HBr; 20 = H. T. Morphine and Ethylmorphine HCl; 21 = H. T. Morphine HCl and Scopolamine HBr; 22 = H. T. Ethylmorphine HCl, and Scopolamine HBr.
[c] Appearance: W = white, impart no color to solution; + = slightly discolored, impart pale straw color to solution; ++ = discolored, impart straw color to solution.

The use of mannitol as a diluent stabilized the product as evidenced by the observations that the tablets dissolved readily in water and did not discolor. All tablets stored in a dry atmosphere retained their elegant appearance although their solubility time was less rapid than those stored at atmospheric conditions. Sodium metabisulfite was an effective antioxidant since tablets containing this agent did not discolor. The solutions recommended gave the required adhesiveness to the tablets and endowed the tablet mixtures with the desired molding properties.

The results of this experiment appear in Tables I, II, III, and IV.

It was concluded that the use of mannitol as diluent, incorporation of sodium metabisulfite into formulas containing lactose as diluent, and storage of the tablets in a dry atmosphere were important factors for maintaining elegant tablets. The apparent deterioration of the tablets studied was not related to the degree of discoloration. There is considerable doubt whether the deterioration encountered in this work is significant, since decrease in alkaloidal strength of the tablets was small regardless of storage or degree of discoloration.

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Studies on Tolerance and Cross-Tolerance to Nostal (Propallylonal; Isopropyl-*beta*-bromallyl Barbituric Acid)*,†

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Nostal in two concentrations and Alurate, Sigmodal, and Ipral in concentrations corresponding to the lower concentration of Nostal (100 mg./Kg. of powdered laboratory chow) were fed during 28 days. Alurate, Ipral, and the higher concentration of Nostal gave complete protection against delayed death. Ipral was most effective; no ipralized rat lost its righting reflex when Nostal was given. The effect of feeding low concentrations of Nostal and Ipral, respectively, in the diet for 37 days was compared against the effects of 60, 90, and 135 mg. of Nostal per Kg. Again, Ipral was more effective in preventing loss of the righting reflex, acute and delayed death. Feeding Ipral in the chow for two days produced marked, and for four days, very marked tolerance to Nostal. The lowest effective concentration of Ipral in the diet was 38 mg./Kg. of chow when fed for ten days. Cross-tolerance to Nostal induced by administering Ipral sodium largely disappeared in eight days. Administration of isopropyl-*beta*-chlorallyl barbituric acid in the diet for ten days induced a marked cross-tolerance to Nostal.

THE ADMINISTRATION of Nostal (isopropyl-*beta*-bromallyl barbituric acid) to the rat may cause not only acute depression but may be followed by acute or delayed death (1, 2). The present study was undertaken to determine whether or not repeated administration of Nostal and related barbiturates produced tolerance, taking into consideration not only the sleeping time, but doses that caused acute or delayed death. In a previous study it had been observed that tolerance can be induced by injection of six semiweekly doses of Nostal (10 mg./Kg.) insufficient to cause discernible depression of the central nervous system (3). Furthermore, not only has a similar tolerance been reported with intravenous administration of Nostal in the dog by Green and Koppanyi, but they also report that hexobarbital-tolerant dogs are tolerant to thiopental and Nostal, and that Nostal-tolerant dogs are tolerant to hexobarbital, thiopental, and barbital (4). In their extensive critical review of the controversy concerning tolerance and cross-tolerance with barbiturates, these authors emphasize that, in their opinion, possible variation in absorption rate must be ruled out before claims for true tolerance can be considered valid. We shall return to certain phases of this problem after presentation of our experimental data.

The objectives of the present series of experiments are: (*a*) to ascertain if any relationship exists between chemical structure and ability to induce cross-tolerance; (*b*) to find out if tolerance can be induced by including various barbiturates in the food; (*c*) to determine the age at which delayed death begins to occur with Nostal; (*d*) to determine the degree of tolerance and cross-tolerance that may be developed; (*e*) to ascertain the concentrations of Ipral sodium in the food required to induce perceptible and maximum cross-tolerance; (*f*) to estimate the time needed to induce both minimal and optimal degrees of cross-tolerance; (*g*) to establish the duration of cross-tolerance; and (*h*) to compare the effects of Nostal by intravenous and intraperitoneal administration in rats in which cross-tolerance has been induced.

EXPERIMENTAL

General.—Unless otherwise specified, the rats used in these studies were bred from Wistar strain rats. The diet consisted of Purina Laboratory Chow and water. The animal quarters were kept at a temperature of approximately 25°. Following administration of Nostal, a rat was considered to be asleep when the righting reflex had been abolished and the animal was lying quietly on its side. Recovery was considered to be complete when a rat promptly retracted either hind leg from an extended position. In order to record incidences of delayed death, each group of rats was observed for five days following the final administration of Nostal. All barbiturates except Nostal were supplied to us as

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The first two experiments (Tables I–IV) are taken from the Master's thesis of Carl C. Riedesel and two experiments (Tables V and VII) from the Bachelor's thesis of Francis A. Robidoux.

the sodium salts. Shortly before administration, Nostal was converted to sodium Nostal by the addition of an appropriate amount of sodium hydroxide solution.

Tolerance and Cross-Tolerance Following Subcutaneous Injections of Barbiturates.—The first tests were carried out upon nine groups, each consisting of 12 albino rats. The rats in four of the groups were male and those comprising the remaining five groups were female. Because of the greater sensitivity to Nostal of the females, a separate control group was employed for each sex. The control groups were injected with 0.9% sodium chloride solution at the same time the remaining test groups were injected with barbiturates. Three barbiturates, Nostal, Ipral (probarbital; isopropyl ethyl barbituric acid), and Eunarcon (N-methyl isopropyl-*beta*-bromallyl barbituric acid) in equimolecular subhypnotic doses (as indicated in the footnote to Table I) were administered subcutaneously twice weekly to the male rats. The females similarly received Nostal, Sigmodal (2-amyl bromallyl barbituric acid), Dial (diallyl barbituric acid), and Alurate (aprobarbital; isopropyl allyl barbituric acid); see footnote to Table II. On the third day after the sixth injection of each barbiturate, 60 mg./Kg. of Nostal (males, intraperitoneally; females, subcutaneously) were given to each rat in these series. The same dose was given to each control rat.

The results of these tests are summarized in Tables I and II. The results obtained with the male rats show that not only did the injections of Nostal, Eunarcon, and Ipral reduce the acute depression time following the final dose of Nostal and in the order given, but they also protected the animals against delayed death. Although Ipral was most effective in reducing the depression time, Nostal was equally effective against delayed death; Eunarcon seemed somewhat less effective, though the number of rats is too small to draw a final conclusion in this respect.

In the case of the female rats, considerable variation is noted in the ability of the barbiturates studied, both in reducing the sleeping time and in protective power against delayed death. Thus, although some decrease in sleeping time is seen following either Sigmodal or Dial, the incidence of delayed death is not altered. Statistical analysis shows that the reduction in acute effects is not significant with Sigmodal ($t = 1.71$; $p = 0.1$), whereas with Dial it probably is significant ($t = 2.02$; p about 0.06). With either Alurate or Nostal the sleeping time is reduced to about half of the control value, yet Nostal premedication is more effective in preventing delayed death. A considerably larger number of rats would be required to determine whether the reduction in sleeping time closely parallels protection against delayed death. It is also of interest to note that the female control and Nostal-premedicated groups of rats slept much longer than similar male groups, although the incidence of delayed death was practically the same.

TABLE I.—COMPARISON OF INCIDENCE OF DELAYED DEATH AND SLEEPING TIME FOLLOWING ADMINISTRATION OF AN HYPNOTIC DOSE OF NOSTAL IN MALE RATS GIVEN PRELIMINARY SUBHYPNOTIC MEDICATION WITH EUNARCON, IPRAL SODIUM, AND NOSTAL[a]

Preliminary Treatment	Average Recovery Time, Min.	Mortality
0.9% NaCl solution	104	11/12
Eunarcon	52	4/12
Ipral sodium	25	2/12
Nostal	66	1/12

[a] Preliminary treatment consisted of 6 successive injections, one every 3–4 days, for 3 weeks: Nostal, 10.0 mg./Kg.; Ipral sodium, 7.62 mg./Kg.; and Eunarcon, 11.25 mg./Kg.; followed on the 21st day by 60 mg./Kg. of Nostal.

TABLE II.—COMPARISON OF INCIDENCE OF DELAYED DEATH AND SLEEPING TIME FOLLOWING SUBCUTANEOUS ADMINISTRATION OF AN HYPNOTIC DOSE OF NOSTAL IN FEMALE RATS GIVEN PRELIMINARY SUBHYPNOTIC MEDICATION WITH SIGMODAL SODIUM, DIAL SODIUM, SODIUM ALURATE AND NOSTAL[a]

Preliminary Treatment	Average Recovery Time, Min.	Standard Error	Mortality
0.9% NaCl solution	260	26	11/12
Sigmodal sodium	204	8.8	10/12
Dial sodium	199	15.8	10/12
Sodium Alurate	131	...	6/12
Nostal	144	...	0/12

[a] Preliminary treatment consisted of 6 successive subcutaneous injections, one every 3–4 days, for 3 weeks: Nostal, 10.0 mg./Kg.; sodium Alurate, 8.02 mg./Kg.; Dial sodium, 7.90 mg./Kg.; and Sigmodal sodium, 11.7 mg./Kg.; followed on the 21st day by Nostal, 60 mg./Kg.

Age Factor in Effects of Nostal.—In previous work by Holck and Mathieson (5) it was reported that young rats are more tolerant to pentobarbital and build up tolerance to this drug more readily than adult animals. To ascertain the age at which the rat becomes suitable for studies on incidence of delayed death following administration of Nostal, we confined our next experiment to three groups of young rats.

In the first phase of this study, varying doses of Nostal were injected subcutaneously into 30-day-old rats. A 1% solution was used to insure greater accuracy of measurement. The results are tabulated in Table III. With doses of 40 and 50 mg./Kg. hypnosis was not induced in any of the rats. This was true for both males and females, of which there was an equal number in each group. With 62.5 mg./Kg. the average time of recovery from acute depression was 1.0 hour in either sex; the only rat which recovered from acute depression only to die later was a female. The period of acute depression, 90 minutes, was the longest recorded for any of the rats with this dose. Thus, one-month-old rats of either sex were found to be much more resistant to Nostal than adult rats.

TABLE III.—COMPARISON OF INCIDENCE OF DELAYED DEATH IN ONE-MONTH-OLD RATS AT THREE DOSAGE LEVELS[a]

Nostal, Mg./Kg.	No. Rats Used	Mortality
40	14	0/14
50	14	0/14
62.5	14	1/14

[a] Nostal was administered subcutaneously and the rats were observed for five days. Each group consisted of 7 males and 7 females.

In the second phase of this study, one group of 44-day-old rats and one group of 67-day-old rats were injected subcutaneously with a 2% solution of Nostal. With a dose of 50 mg./Kg. none of the rats lost the righting reflex although 17 in the 44-day-old group were females and all in the 67-day-old group were females. However, it can be seen, from Table IV, that female rats two months old or older are much more prone to die several days after Nostal medication than younger rats. Holck and Cannon (2) had previously reported that even in the case of adult rats delayed death may occur with subhypnotic doses, although death was not as frequent as found here.

TABLE IV.—COMPARISON OF INCIDENCE OF DELAYED DEATH IN RATS OF THREE DIFFERENT AGES, RECEIVING 50 MG./KG. OF NOSTAL SUBCUTANEOUSLY[a]

Age in Days	No. Rats Used	Mortality
30	7 males 7 females	0/14
44 ± 1	7 males 17 females	1/24
67 ± 2	15 females	12/15

[a] The 30- day-old rats were injected with a 1% solution and the two groups of older rats were injected with a 2% solution.

Induction of Tolerance and Cross-Tolerance by Oral Barbiturate Administration.—The objectives of these tests were to ascertain if oral administration of barbiturates would be as effective in inducing higher resistance as the injection technique so far employed, and to confirm the values showing relative efficiency of the barbiturates hitherto found effective in decreasing the effects of a single, large dose of Nostal. Six groups of 12 male rats each were studied. The barbiturates were thoroughly mixed with powdered Purina Laboratory Chow and the concentrations based upon the findings obtained in a preliminary test showing the daily food consumption was approximately 50 Gm./Kg. of rat. In addition to Nostal, administered at two concentration levels, Alurate, Sigmodal, and Ipral were admixed with the food. The feeding was continued for 28 days. The results are presented in Table V.

Although the food consumption varied from 50 Gm./Kg. per day in the Sigmodal group to 59 in the Ipral group, the amount of barbiturate consumed per day came to within ±8% of what would have been the case had all rats eaten 55 Gm./Kg. per day, as was the case in the group receiving the lower concentration of Nostal. Again Ipral was most effective in raising the resistance, in that none of the rats lost the righting reflex and all survived the final Nostal medication. Sigmodal was least effective in either respect, and the higher concentration of Nostal a little better than the lower one. Alurate also was very effective. Thus, again, Ipral was more satisfactory than Nostal, Sigmodal quite uncertain, and Alurate gave better protection against delayed death than in the experiments in which the barbiturates were administered by repeated injections.

In a second phase of our study of tolerance and cross-tolerance to Nostal by administering various barbiturates in the food, six groups of 3- to 5-month-old male rats were studied. The feeding period was limited to 10 days and the dose of Nostal increased by 10% to 66 mg./Kg. In addition to Nostal and Ipral sodium we included administration of isopropyl chlorallyl barbituric acid, the chlorine homolog of Nostal, and isopropyl acetonyl barbituric acid, the supposed first decomposition product of Nostal, in the food. In case of Ipral sodium and of the chlorine homolog, the concentration was made to correspond on an equimolecular basis to that of Nostal. However, only the lower concentration of isopropyl acetonyl barbituric acid corresponded to that of Nostal. In view of the fact that this substance is much weaker in hypnotic power than Nostal (2), we included a concentration five times as great. The results are tabulated in Table VI. As may be noted, Ipral sodium again was more effective than Nostal itself in inducing tolerance to Nostal. Cross-tolerance to Nostal was induced by the chlorine homolog, but it was not quite as efficient as Nostal as judged by the average recovery time. With isopropyl acetonyl barbituric acid no cross-tolerance was seen with the lower concentration; as to the higher concentration, the experiment was unsatisfactory in that every rat showed a severe loss in body weight and one of them died on the tenth day. In view of the fact that an indication of cross-tolerance seemed to exist, an intermediate concention of 156.4 mg./Kg. for twice the length of time was tested (see Table X).

Relative Effectiveness of Nostal and Ipral Feeding in Raising the Resistance to Larger Nostal Doses.—In our second series of feeding experiments only Nostal and Ipral were administered in the diet of male rats, but the final Nostal dose was given at three levels; each successive dose increase was by 50%. This enabled us to compare the effectiveness of these two barbiturates not only in regard to lower-

TABLE V.—COMPARISON OF RECOVERY TIME AND INCIDENCE OF DELAYED DEATH FOLLOWING SUBCUTANEOUS ADMINISTRATION OF 60 MG. OF NOSTAL/KG. TO MALE ALBINO RATS KEPT 28 DAYS ON POWDERED PURINA LABORATORY CHOW CONTAINING VARIOUS BARBITURATES

Diet	Drug, Mg./Kg. of Food	Average Recovery Time, Min.	Recovery Time as Per Cent of Controls	No. Delayed Deaths	Mg. of Barbiturate/-Kg. of Rat/Day
Controls	Food only	121	100	6/12	...
Nostal	100	44	36	2/12	5.5
Nostal	200	35	29	0/12	10.6
Sodium Alurate	160.5	53[a]	44	0/12	8.7
Sigmodal sodium	226.4	112	93	5/12	11.3
Ipral sodium	152.3	Tranquilized only	...	0/12	8.9

[a] Average taken from ten animals, as two in the group were only tranquilized.

TABLE VI.—COMPARISON OF RECOVERY TIME AND INCIDENCE OF DELAYED DEATH FOLLOWING INTRAPERITONEAL ADMINISTRATION OF 66 MG. OF NOSTAL/KG. TO MALE ALBINO RATS SUBSEQUENT TO TEN-DAY DIET OF POWDERED PURINA LABORATORY CHOW CONTAINING VARIOUS BARBITURATES

Diet	Drug, Mg./Kg. of Food	Average Recovery Time, Min.	Recovery Time as Per Cent of Controls	No. Delayed Deaths	Mg. of Barbiturate/-Kg. of Rat/Day
Control	Food only	152	...	8/12	...
Nostal	100	52	34	2/11[a]	6.0
Ipral sodium	76.2	39 (2)[b]	...	1/12	5.1
Isopropyl chlorallyl barbituric acid	84.3	73	48	3/12	5.2
Isopropyl acetonyl barbituric acid	78.2	161	106	7/12	5.1
Same	391	115	76	2/11[c]	17.9

[a] One rat died acutely; it showed gross pathology of the lungs.
[b] Ten rats did not lose the righting reflex; average is based on the two that did.
[c] One rat died during the last day of feeding; the remainder lost an average of 25.5% in body weight during the 10-day feeding period, whereas the other five groups gained slightly in weight.

ing the depression time and to preventing delayed death, but also to diminishing the number of acute deaths. To make certain of full effects, the feeding was continued for 37 days with the six groups of rats receiving barbiturates in the diet. The results are presented in Table VII.

Nostal was fed in the concentration found to be most effective in the previous experiment and was again found less effective than an equimolecular concentration of Ipral in the food. This is strikingly demonstrated by the reduction of the acute depression time of Nostal, especially with the highest dose, and also by the prevention of delayed death. The greater efficiency of Ipral is not clearly shown in comparing acute deaths; higher final doses of Nostal and many more rats would be needed to show if any real difference exists in this respect. In all cases both premedications significantly decreased the acute sleeping time and reduced incidences of both acute and delayed death as compared with the control.

Time Required to Induce Cross-Tolerance to Nostal.—The purpose of this experiment was to determine the length of time required to produce both demonstrable and optimum cross-tolerance to Nostal by feeding suitable concentrations of Ipral sodium in the diet. Six groups of Sprague-Dawley albino male rats, 6–7 months old, were fed a diet containing 152.3 mg. of Ipral sodium/Kg. of powdered Purina Laboratory Chow. This diet was fed for periods of 2, 4, 8, 16, 22.6, and 32 days respectively. In view of the fact that a large number of days would intervene between 16 and 32 days, a period of 22.6 days was added. This represents an increase in duration of 41.3% or one-half the logarithm of two. In order to avoid the use of more than one control group, the feeding was arranged so that all of the rats could be injected with Nostal on the same day. On that day each rat was given 73.5 mg. of Nostal/Kg. intraperitoneally, after which observations were made on recovery time, and acute and delayed deaths. The room temperature during the acute depression phase was 25–26°. A summary of the results is presented in Table VIII.

The very high t factor of 8.9, as well as the complete prevention of both acute and delayed deaths from Nostal, indicate that some degree of cross-tolerance can be built up in less than two days. However, considerably longer periods are required to develop optimum or maximum tolerance. Tolerance increases scarcely at all with Ipral medication beyond four days, except that after long feeding more rats no longer lose the righting reflex when Nostal is given. Consequently, comparisons among various drugs as to their capacity to induce cross-tolerance may profitably be carried out in 10-day tests. The results also indicate that the concentration of Ipral sodium which was used is too high for prolonged experiments in that the intake gradually decreased, the longer it was fed. Because of this, a lower concentration may be more suitable.

TABLE VII.—COMPARISON OF RECOVERY TIME, AND INCIDENCES OF ACUTE AND DELAYED DEATH FOLLOWING SUBCUTANEOUS ADMINISTRATION OF SELECTED DOSES OF NOSTAL TO MALE ALBINO RATS KEPT 37 DAYS ON POWDERED PURINA LABORATORY CHOW WITH AND WITHOUT NOSTAL OR IPRAL SODIUM

Group	Diet	Nostal Mg./Kg. of Rat	Drug, Mg./Kg. of Chow	Drug, Mg./Kg. of Rat per Day	Average Recovery Time, Min. and No. of Rats[a]	Acute Death	Delayed Death
A	Food only	60	...	...	101 (12)	0/12	10/12
	Nostal	...	200	10.7	46 (8)	0/12	0/12
	Ipral sodium	...	152.3	9.3	0 (12)[b]	0/12	0/12
B	Food only	90	...	...	239 (8)	4/12	6/12
	Nostal	...	200	10.3	69 (10)	0/12	1/12
	Ipral sodium	...	152.3	9.3	34 (3)	0/12	0/12
C	Food only	135	...	...	306 (2)	10/12	2/12
	Nostal	...	200	11.1	131 (11)	1/12	5/12
	Ipral sodium	...	152.3	8.2	0 (12)[b]	0/12	2/12

[a] Figures in parentheses represent number of rats upon which the average is based; the lower number is due either to acute deaths or failure to produce loss of righting reflex.
[b] Tranquilized only.

TABLE VIII.—EFFECT OF FEEDING IPRAL SODIUM FOR VARYING PERIODS OF TIME UPON THE RESPONSE OF THE MALE ALBINO RAT TO NOSTAL[a]

Group	No. of Rats	Average Recovery Time, Min.	Standard Error	*t* Factor	Acute Deaths	Delayed Deaths	Mg. of Ipral sodium/Kg. of Rat/Day	Total
Control	10	215 (4)[b]	18.3	....	6	4	...	...
2 days	10	45 (10)	5.4	8.9 (C *vs.* 2 D)	0	0	7.9	16
4 days	10	16 (10)	2.1	5.0 (2 *vs.* 4 D)	0	0	7.8	31
8 days	10	15 (10)	1.7	....	0	0	6.5	52
16 days	10	15 (8)	...	....	0	0	6.5	102
22.6 days	9	13 (4)	...	....	0	0	6.5	147
32 days	10	14 (6)	...	....	0	0	5.8	186

[a] 73.5 mg. of Nostal (given as the sodium salt) was injected intraperitoneally into adult albino Sprague-Dawley Rats after maintaining these for the designated periods of time on powdered Purina Laboratory Chow containing 152.3 mg. of Ipral sodium/Kg. of food.
[b] Figures in parentheses represent number of rats upon which the average is based; the lower number is due either to acute deaths or failure to produce loss of righting reflex.

In view of the fact that no cross-tolerance was apparent with isopropyl acetonyl barbituric acid, the supposed first decomposition product of Nostal, when mixed with the food in a concentration equimolecular to that of Nostal used in this experiment, and because the rats did not thrive upon five times that concentration in the food, a 20-day feeding experiment with only two and a half times the equimolecular concentration (156.4 mg./Kg. of food) was added. It was combined with later tests on duration of cross-tolerance and the results are included in Table X.

With this concentration of isopropyl acetonyl barbituric acid in the food and the longer duration of feeding, cross-tolerance was induced. However, even with this rather high concentration it was not as effective as the lower concentration of Ipral sodium in the food, either in reducing the average depression time or in preventing delayed death. It may also be noted that—in contrast with twice the present concentration previously tried—with the present concentration of isopropyl acetonyl barbituric acid, the rats gained weight to the same extent as the controls.

Dietary Concentration of Ipral Required to Induce Cross-Tolerance to Nostal.—In the preceding experiment we ascertained that 152.3 mg. of Ipral sodium/Kg. of food will induce a marked cross-tolerance to Nostal in two days and a very marked one in four days. In this experiment the following concentrations of Ipral sodium were fed for ten days prior to Nostal medication to determine the minimum effective concentration: 19.05, 38.1, 76.2, 152.3, and 304.6 mg./Kg. of powdered Purina Laboratory Chow, respectively. Ten days of feeding a medicated diet was chosen for reasons given in the preceding discussion. Intraperitoneal administration of 60 mg. of Nostal/Kg. of rat was selected because with this dose acute death was unlikely, but delayed death probable in the majority of the rats. The temperature during the acute depression period was 25–26°. In the case of nine rats which had lost body weight, no acute deaths occurred and they showed no signs of abnormality. However, seven of them recovered slowly from Nostal as compared with others in their groups, and five died later on. These rats were distributed as follows: Group A, 1; B, 1; C, 2; D, 3; E, 1; F, 1; the losses in body weight can neither be ascribed to the medication with Ipral sodium, nor—as judged by the food-intake in this series—to any distaste for the medicated diet. The experimental data are summarized in Table IX.

The results of these experiments show, primarily, that when the feeding period is limited to ten days, rats consumed approximately the same amount of food in proportion to their body weights, regardless of the concentration of Ipral sodium in the food. They also show that although the number of delayed deaths is distinctly diminished with the concentration of 38.1 mg. of Ipral sodium/Kg. of food, the recovery time of the test group as compared with that of the controls is not quite significant; the *t* factor is only 1.60 (p about 0.13).

TABLE IX.—EFFECT OF FEEDING VARYING CONCENTRATIONS OF IPRAL SODIUM FOR TEN DAYS UPON THE RESISTANCE OF THE ALBINO RAT TO NOSTAL[a]

Group	Ipral Sodium, Mg./Kg. of Purina Laboratory Chow	No. Rats	Average Recovery Time, Min.	Delayed Deaths	Ipral Sodium, Mg./Kg. of Rat Per Day
A	Control	12	139	9	...
B	19.05	12[b]	127	8	1.1
C	38.1	12	97	2	1.9
D	76.2	11[c]	83 (9)[d]	4	3.6
E	152.3	11	34 (3)	0	7.7
F	304.6	12	28 (1)	0	16.1

[a] At the end of 10 days, 60 mg. of Nostal/Kg. of rat was administered intraperitoneally. Male rats 4–12 months old were employed in this experiment.
[b] One rat had lost considerably in weight.
[c] Two rats had lost considerably in weight.
[d] Figure in parentheses represents number of rats that lost the righting reflex, thus the number upon which the average is based.

TABLE X.—(*a*) DURATION OF CROSS-TOLERANCE TO NOSTAL AND (*b*) THE EFFECT OF ADMINISTERING ISOPROPYL ACETONYL BARBITURIC ACID ON THE RESISTANCE OF THE ALBINO RAT TO NOSTAL

Group	Food or Food with Ipral Sodium (Underscored) in No. of Days[a]	Average Recovery Time, Min.	Standard Error	*t* Factor	*p*	Delayed Deaths	Percentage Gains in Body Weight
A	52	94	6.42	...	...	4	57
B	20–32	99	8.36	...	...	5	56
C	16–20–16	103	6.96	...	...	5	63
D	24–20–8	76	7.32	1.85 (A *vs.* D)	0.09	2	58
				2.67 (C *vs.* D)	0.02		
E	32–20	27 (2)[b]	...	...	...	0	59
	Food or Food with Isopropyl Acetonyl Barbituric Acid (Underscored) in No. of Days[c]						
F	32–20	69	5.28	3.05 (A *vs.* F)	0.01	2	62

[a] 76.15 mg./Kg. of food. Each group consisted of twelve 3½-to 5-month-old male rats and the final dose of Nostal was 60 mg./Kg., administered intraperitoneally.
[b] Two rats only lost the righting reflex.
[c] 156.4 mg./Kg.

With a feeding period of only ten days of 76.2 mg./Kg. of food, delayed death was not completely prevented, although the *t* factor for the acute recovery times was 2.87 ($p = 0.01$). Furthermore, this *t* factor does not fully express the total difference because two rats which did not lose their righting reflex are not included.

Using 152.3 mg. of Ipral sodium/Kg. of food not only completely prevented delayed death, but also was distinctly more effective in reducing the acute depression time, in that eight of the eleven rats tested never lost the righting reflex.

Duration of Cross-Tolerance to Nostal.—In order to determine persistence of cross-tolerance to Nostal after ending the administration of Ipral sodium in the food, an experiment was carried out in which every rat was fed for 20 days with a concentration of 76.15 mg. of Ipral sodium/Kg. of food. Feeding periods were timed in such a way that the final Nostal administrations, which were all made on the same day, occurred 8, 16, and 32 days, respectively, after discontinuing the addition of Ipral sodium to the food. In our earliest experiments when Ipral sodium was administered twice weekly and Nostal given three days after the sixth injection, cross-tolerance was marked. Consequently, no period of less than eight days was employed in the present study. A control group, whose food contained no Ipral sodium, was included. The final dose of Nostal was 60 mg./Kg. of rat, administered intraperitoneally. The rats were all males and were 3½ to 5 months old when Nostal was administered. The results are tabulated in Table X.

The results of this experiment indicate that although the usual marked cross-tolerance was built up by administering Ipral sodium in the food for 20 days immediately preceding the Nostal medication, the cross-tolerance had largely disappeared eight days after discontinuing Ipral medication. This conclusion is based on both average depression time and the number of delayed deaths. Although the recovery time is somewhat longer than with the controls in the group in which Ipral administration was discontinued 16 days before Nostal was given, the difference is not significant as seen by the probability factor. It is somewhat puzzling that there should occur what seems to be a significant difference between the groups in which Ipral administration was discontinued eight and sixteen days, respectively, before the Nostal medication. The rapid disappearance of tolerance may be due in part to the fact that the food contained a rather low concentration of Ipral sodium. The final column in Table X indicates that this low concentration is harmless so far as growth is concerned, in that the Ipral-treated rats grew at about the same rate as the controls.

Cross-Tolerance with Intravenous Nostal Administration.—Because increased tolerance to Nostal by preliminary administration of Ipral sodium could possibly be due to a slowing of the absorption of

TABLE XI.—DEGREE OF CROSS-TOLERANCE TO INTRAVENOUSLY ADMINISTERED NOSTAL INDUCED BY ADMINISTERING IPRAL SODIUM IN THE FOOD FOR 21 DAYS OR FOR 28 DAYS WITH TWO ADDITIONAL SUBCUTANEOUS INJECTIONS OF IPRAL SODIUM[a]

Group	No. Rats	Pretreatment[b]	Average Recovery Time, Min.	Standard Error	*t* Factor	Delayed Deaths
A	8	None	143	12.2	....	4
B	8	Ipral in food	63	9.3	5.2 (A *vs.* B)	2
C	5	Ipral in food plus two injections	40	5.4	7.5 (A *vs.* C)	1

[a] The female rats were 3½ to 5 months old.
[b] The concentration of Ipral sodium in the food was 76.15 mg./Kg.; the subcutaneous dose of Ipral sodium was 18 mg./Kg. (20% of the anesthetic dose) of rat; the injections were made during the fourth week of feeding and given five and three days, respectively, before Nostal medication. The dose of Nostal was 50 mg./Kg. of rat.

Nostal, the following experiment was added to rule out such a possibility. Following the general plan of Green and Koppanyi (4), we administered the 50 mg. of Nostal/Kg. of rat by vein. The injection was made into a femoral vein which had been quickly exposed under local anesthesia with ethyl chloride; following application of Merthiolate solution, the incision was closed with a wound clip. Three groups of female rats were employed; the age of the rats was $3^1/_2$ to 5 months. The control group received no Ipral sodium; the second group received 76.15 mg. of Ipral sodium/Kg. of food for 21 days prior to the Nostal injection. The third group was given such medicated food for 28 days plus a subcutaneous injection of 18 mg. of Ipral sodium/Kg. of rat on the fifth and third days, respectively, before Nostal was administered; 18 mg. of Ipral sodium is 20% of the anesthetic dose. The results are tabulated in Table XI.

The marked decrease in recovery time clearly shows that true tolerance to Nostal can be established by preliminary medication with Ipral sodium. With the absorption factor ruled out, the very high t factors give good evidence to support this statement. It is also noteworthy that the concentration of Ipral sodium used in the food apparently did not lead to maximum tolerance, as indicated by the further raising of tolerance by additional subcutaneous injections of Ipral sodium.

DISCUSSION

In this series of experiments we have demonstrated that tolerance and cross-tolerance to Nostal can readily be induced in rats by injecting several subhypnotic doses of Nostal or various more or less closely related barbiturates for a suitable length of time before the final hypnotic dose of Nostal. Similarly, tolerance may be induced by adding the barbiturates to the food of the rat for a suitable length of time prior to injection of Nostal. All barbiturates, however, were not equally effective in inducing cross-tolerance or tolerance. Dial sodium and Sigmodal sodium were quite ineffective under the conditions employed here, whereas Nostal, sodium Alurate, Eunarcon, and especially Ipral sodium—all containing the isopropyl group—were very effective.

Because incidence of delayed death was one of our major criteria in judging the degree of tolerance only mature rats were used. Delayed death is not likely to follow rather large doses of Nostal in rats less than six weeks old; young rats also are more resistant to the acute effects of Nostal. That cross-tolerance can be built up to a considerable degree within two, and especially four days, is suggestive of previous tests concerning tolerance in the case of pentobarbital in which case tolerance can readily be observed after a short series of injections given at 90-minute intervals to adult male rats or young rats of either sex (3, 5). The results in the present experiments with Nostal are similar in general to those of Green and Koppanyi who studied the effects of barbiturates in dogs. In their tests, Nostal tolerance induced by preliminary small daily doses of Nostal was distinct in two to four days and maximum in about a week (4). Our results also agree with the early report by Fitch that in rabbits the recovery time with Nostal, Neonal, or Amytal was shortened by almost 50% after two or three daily doses of any one of these substances (6). Likewise, they agree with the marked tolerance to Nostal observed by Oettel and Krautwald after a few daily hypnotic doses of this drug to dogs (7).

Our results also show a decrease in the proportion of rats which died following recovery from the acute effects of Nostal when the recovery time was materially shortened in consequence of administering preliminary subhypnotic doses of various barbiturates. However, it is not possible to show a direct relationship between the average recovery time and incidence of delayed death. Thus, in case of the normal males which recovered in 104 minutes, 11 out of 12 rats died during the next five days, whereas, in case of female rats which were pretreated with subhypnotic doses of Nostal, the average recovery time was 144 minutes, yet none of the 12 rats died during the five days following administration of 60 mg./Kg. of Nostal (Tables I and II). On the other hand, we have found it easy to increase both the average recovery time and the incidence of acute deaths by partial hepatectomy or by carbon tetrachloride administration without raising the incidence of delayed death; these experiments will be published separately (8). Thus, although detoxification of, or resistance to, Nostal plainly is altered in some respects by partial hepatectomy or administration of hepatoxic agent, such change does not extend to all actions of Nostal in the rat. It is of interest to note here that Green and Koppanyi reported that in hexobarbital-tolerant dogs, tolerance did not include several peripheral actions (4). This was in agreement with reports by Nowak that in hexobarbital-tolerant dogs, some, but not all circulatory, responses were modified (9, 10).

The fact that Ipral sodium was clearly superior to Nostal in inducing tolerance to Nostal is another interesting phenomenon. It is of further interest to note that cross-tolerance to Nostal could be induced in dogs, studied by Green and Koppanyi, by preliminary administration of either hexobarbital or thiopental to such an extent that even intravenously administered Nostal would not induce sleep (4). These authors also reported that thiopental recovery time was more rapid in hexobarbital-tolerant dogs than in thiopental-tolerant animals. We did not use hexobarbital or thiopental in our studies, but in case of our Ipral-tolerant rats we did markedly shorten (though not completely prevent) sleep induced by giving 50 mg./Kg. of Nostal by vein. This dose is considerably greater than the 30 mg./Kg. given to the dogs; however, it is difficult to compare doses in different species of animals. In their attempt to elucidate the mechanism of cross-tolerance in dogs, Green and Koppanyi (4) employed Nostal-tolerant dogs to which they administered barbital, a drug which is largely excreted in the urine. No evidence of increased excretion was found, although the recovery time was shortened to 30% of the control value, thus posing a special problem as to the true mechanism of cross-tolerance. Concerning the degree of cross-tolerance possible, Green and Koppanyi cautioned against optimism in expecting an appreciable increase in the fatal dose. Our tests show that in the rat an increase was easily secured not only with regard to delayed, but also acute death, as shown particularly clearly in Table VII.

In regard to persistency of tolerance or cross-tolerance, Green and Koppanyi, Masuda, *et al.* (11), and Gruber and Keyser (12) have reported a rapid disappearance in regard to barbiturates. Our tests confirm their findings in that, although tolerance to Nostal induced by the intermittent injection method was marked on the third day after the last of six injections, cross-tolerance was practically absent eight days after discontinuing the administration of Ipral sodium in the food.

It seems of further interest to note one aspect of the work on induction of cross-tolerance to Nostal by isopropyl acetonyl barbituric acid. This is one of the early decomposition products of Nostal according to Boedecker and Ludwig (13); the Br has been replaced by an OH group, and subsequent rearrangement to the keto-form has taken place. This substance is slower in action and the anesthetic dose is about eight times that of Nostal (2, 13). However, when rats were fed 312.8 mg./Kg. of isopropyl acetonyl barbituric acid in the food for a ten-day period, or only two and one-half times the molecular equivalent of Nostal (200 mg./Kg. of food), all the rats lost heavily in weight, whereas, in the case of the Nostal feeding, weight was well maintained during a 37-day test. Also, although isopropyl acetonyl barbituric acid contains the isopropyl group, it is feeble in its ability to induce cross-tolerance to Nostal, as compared to the other four barbiturates which contain this group and are included in this study.

Finally, our results are not in agreement with the findings of Gruber and Keyser (12), who reported that although tolerance was built up in rabbits to Butisol, pentobarbital or Seconal, as indicated by a marked decrease in sleeping time, the LD_{50} was essentially unchanged. The drugs were administered intravenously at a uniform rate. In our studies, which were limited mainly to subcutaneous and intraperitoneal injections, it is repeatedly shown that whenever tolerance or cross-tolerance effects a marked reduction in sleeping time, there is a concomitant sharp decrease in the number of animals which die acutely or during the next few days.

SUMMARY

1. Tolerance to Nostal, as judged by duration of depression and number of delayed deaths, was induced by administering six subhypnotic doses of Nostal, sodium Alurate, Eunarcon, or Ipral sodium, one every three to four days during three weeks. Ipral sodium and Nostral were most effective. No certain cross-tolerance was induced by preliminary medication with Dial sodium or Sigmodal sodium.

2. Following subcutaneous administration of 50 mg./Kg. of Nostal, delayed deaths did not occur in 30-day-old rats, rarely in 44-day-old rats, but frequently in 67-day-old rats.

3. When powdered food containing low concentrations of Nostal, sodium Alurate, Ipral sodium, or Sigmodal sodium was fed for 28 days, tolerance to Nostal was induced in case of the first three barbiturates. It was most marked with Ipral sodium.

4. Powdered food containing Nostal (200 mg./Kg.) or Ipral sodium (153.2 mg./Kg.) was fed for 37 days. On the thirty-eighth day Nostal was administered at three different dosage levels. Ipral sodium was much more effective than Nostal in building up tolerance to the latter at all three dosage levels, as manifested by markedly shorter recovery times as well as fewer delayed deaths.

5. Cross-tolerance to Nostal was induced by administering the supposed first decomposition product of Nostal, isopropyl acetonyl barbituric acid (156.4 mg./Kg.), in the food for 20 days. It was not as effective as Ipral sodium. A lower concentration (62.6 mg./Kg.) did not induce cross-tolerance, and when given twice the first-mentioned concentration (312.8 mg./Kg.), the rats did not thrive.

6. By feeding powdered food containing Ipral sodium (152.3 mg./Kg.) for two days, marked cross-tolerance to Nostal was induced; this cross-tolerance was significantly greater when feeding was carried out for four days; still longer periods of feeding increased the cross-tolerance but slightly.

7. The lowest concentration of Ipral sodium in the food which induced cross-tolerance to Nostal when fed for 10 days was 38.1 mg./Kg.; with 19.05 mg./Kg., cross-tolerance could not be demonstrated.

8. Cross-tolerance to Nostal induced by administering Ipral sodium (76.15 mg./Kg.) in the food for 20 days largely disappeared in eight days and completely so in 16 days.

9. That true cross-tolerance was induced is indicated by the fact that the depression time after administration of Nostal by vein was markedly shortened by preliminary administration of Ipral sodium in the food and by additional subcutaneous doses in addition to that administered in the food.

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Cultivation Studies of the Solanaceous Drugs

III. Alkaloidal Content of Mature and Immature Belladonna Leaves*,†

By W. R. BREWER‡ and L. DAVID HINER§

In an attempt to clarify a diversity of opinion in the literature concerning the alkaloidal quality of young, small leaves, and old, large ones from solanaceous plants, an initial study was made of these leaf types taken from belladonna plants. After collection, drying and assaying, the results were subjected to statistical interpretation.

REPORTS in the literature of solanaceous plant culture have shown a diversity of opinion with reference to the correlation of size and age of individual leaves with their alkaloid content. Stulnikov (1) in 1928 claimed that normal, mature leaves assayed higher than the tender, immature leaves. Ten years later, Eby, *et al.*, (2) divided the leaves of the one species of solanaceous plants into two groups—those shorter than 25 mm. and those longer than 25 mm.—and found that the shorter group assayed over twice more than the longer.

An experiment was conducted in an attempt to determine whether leaves of different maturity occuring at the same time on authenticated *Atropa belladonna* plants differed in alkaloid content.

EXPERIMENTAL

At the time of harvest of belladonna, the plants were cut off at about 6 or 8 in. above the ground leaving several large leaves on the remaining part of the stem. By the time new shoots and small leaves developed in abundance, those leaves left on the stem at harvest had grown to a size of from 5 to 7 in. long and $3^1/_2$ to $4^1/_2$ in. wide. Furthermore, they were of a yellowish green color compared to the bright green of the actively growing young leaves and tips. A similar situation develops in untreated plants if no leaves are harvested at the time of flowering. At flower production time there is a noticeable cessation of new leaf formation. During this time, the leaves already present continue to grow and, therefore, stand out markedly from the bright new shoots and leaves which begin to develop again following the setting of fruit. The young leaves collected in the experiment were less than 2 in. long and possessed a bright green color. The old leaves were 5 in. or longer and were less bright. The harvest, in which the basal leaves were left on the stems, was carried out at the time of early flowering and the collection of the enlarged leaves and the small leaves and tips was conducted about three and one-half to four weeks later.

Two collections of old leaves and of actively growing young leaves and tips were made in 1943 from belladonna growing in two separate medicinal gardens. This collection was repeated in 1947 using different plots in a single garden. Each sample represented the material from 30 plants. The leaves were air-dried on trays in a drying loft, then ground to a moderately coarse powder and assayed according to the method of the U. S. P. XII. The results of these assays were subjected to statistical analysis. The difference in the mean assays of the two types of material was tested for significance. The assays of the mature and immature materials from the four collections, the assay means, and the final statistics determining the probability appear in Table I.

TABLE I.—TEST OF SIGNIFICANCE OF DIFFERENCE BETWEEN ALKALOIDAL ASSAY MEANS OF MATURE AND IMMATURE LEAVES OF *Atropa belladonna*
(Assays in Parts per 10,000)

Young Leaves and Tips	Old, Mature Leaves
48	36
38	27
53	19
44	31
45.8 (mean)	28.2 (mean)

t 3.65; D. F. 6; P 2%.

The probability of only 2% that the means could differ as much as they do by mere chance alone is significant evidence that they differ, not by chance, but because the samples differed in their alkaloidal quality.

CONCLUSION

Based on the analysis of the data in Table I, it is safe to conclude that the young leaves of belladonna, as described in the experiment, have a higher percentage alkaloid content than the mature leaves.

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Presented to the Scientific Section, A. PH. A., Atlantic City meeting, May, 1950.
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Cultivation Studies of the Solanaceous Drugs

IV. Time and Space in Solanaceous Culture*,†

By W. R. BREWER‡ and L. DAVID HINER§

In order to determine the best time to fertilize and harvest and the best field spacing in which to plant certain solanaceous species for optimum production and alkaloid yield, belladonna and hyoscyamus plants were chosen for an initial study of these problems. Ammonium sulfate fertilizer was applied at three different stages of belladonna growth; harvests were taken at the same three stages; and plantings of both belladonna and hyoscyamus (biennial variety) were made in three different field spacings.

It is frequently reported in the economic study of field crops such as the cereal grains or the vegetables, that there exists an optimum time for applying soil nutrients or for harvesting a particular plant species, in order to increase its bulk production or its yield of nitrogenous constituents. Likewise, there is frequently found an optimum field spacing which favors maximum production.

Three experiments were designed and carried out in Ohio from 1942 through 1947 in an effort to determine the effect of these factors on the production or alkaloidal content of certain authenticated species of solanaceous drug plants.

EXPERIMENTAL

Time of Fertilizer Application.—In order to determine the optimum stage of plant growth for applying nitrogen fertilizers in an attempt to increase the alkaloidal content of belladonna plants, six plots of 30 individuals each, divided equally in two separate gardens, were enriched with ammonium sulfate at three stages of plant development. The soil of both gardens was relatively low in available nitrates before the start of the experiment. The fertilizer was applied at the rate of 4 lb./100 sq. ft. by spreading it uniformly over the soil, chopping it into the surface with a hoe, and then watering the area. The three times of application were at the premature stage for one-third of the plants, at the flowering stage for another third, and at the fruiting stage for the final third. The time of the "premature stage" fertilizer addition was the second or third week in the field when plants were approximately 6 to 8 in. high. The overground portion of the plants from all plots was harvested three times during the growing season leaving only enough basal leaves on the remaining stem to carry it through to the next harvest. The six harvests from the two plots receiving fertilizer at the premature stage of the plant growth were cured, combined, ground to a moderately coarse powder, mixed, and assayed—as were those from the plots receiving nutrient at the flowering and fruiting stages.

The comparison of alkaloid yields of these plants with a control plot is found in Table I. It shows that the plants which were subjected to the added nutrients early in the season displayed the highest alkaloid assay.

TABLE I.—BELLADONNA ASSAYS[a] OF OVERGROUND PORTIONS OF PLANTS FERTILIZED AT THREE DIFFERENT STAGES OF GROWTH WITH AMMONIUM SULFATE

Treatment	Alkaloidal Assay (Parts/10,000)
Control plots	31
Fertilized in premature stage	37
Fertilized in flowering stage	28
Fertilized in fruiting stage	30

[a] All assays in this report were run according to the method of the U. S. P. XII for total alkaloids.

Time of Harvest.—For the purpose of determining the stage of plant growth in which the highest alkaloidal assay could be obtained from otherwise untreated individuals, the overground portions of six plots of 30 belladonna plants each were collected at three different stages of development. The three were: the premature (plants about 6 to 8 in. in height and without evidence of advanced flower bud development), the flowering, and the fruiting stages.

TABLE II.—ASSAYS OF THE OVERGROUND PORTIONS OF BELLADONNA PLANTS HARVESTED AT THREE DIFFERENT STAGES OF DEVELOPMENT

Stage When Harvested	Alkaloid Yield (Parts/10,000)
Premature	31
Flowering	34
Fruiting	29

Only one such harvest was taken from any plot, so that frequency of harvest and incidence of previous harvest would not enter the experiment as affecting the alkaloidal assay. The six harvests for each stage of development were cured, combined, ground to a moderately coarse powder, mixed, and assayed. Table II, containing the results of the assays, shows that the highest assay was obtained from plants harvested at the flowering stage of development.

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Presented to the Scientific Section, A. PH. A., Atlantic City meeting, May, 1950.
† Part of a dissertation submitted to the Graduate School, Ohio State University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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TABLE III.—BELLADONNA AND HYOSCYAMUS PRODUCTION IN VARIED FIELD SPACING

Plant	Annual Production in Pounds of Air-Dried Weight $\left(\frac{\text{Lb./Plant}}{\text{Lb./Acre}}\right)$ Spacing of 12 In. x 12 In.	Spacing of 18 In. x 18 In.	Spacing of 24 In. x 24 In.
A. belladonna	$\frac{0.164}{6086}$	$\frac{0.173}{4830}$	$\frac{0.158}{2942}$
H. niger	$\frac{0.127}{4756}$	$\frac{0.197}{5501}$	$\frac{0.099}{1858}$
	37,180 plants per acre	27,885 plants per acre	18,590 plants per acre

Field Spacing.—For any field crop, the closest arrangement of plants which will not be detrimental to the yield is a factor of economic importance. In observation of digitalis plants the authors have noticed a difference in dry weight production from plots in which the spacing was varied. This was probably due to the fact that some plots were planted so closely that the leaves of the digitalis overlapped profusely and therefore received less sunlight than those with wider spacing. Furthermore, the soil surrounding closely planted individuals was observed to be more moist, generally, than those with wider spacing. It is a well-known fact that these two factors, light and moisture, affect the carbon-nitrogen ratio in plants thereby influencing such characteristics as size and flower formation.

In order to correlate spacing with dry weight production of solanaceous plants, belladonna (1942 and 1943) and first-year hyoscyamus of the biennial variety (1947) were planted in the field in three plots of 30 plants each. One plot was spaced 12 in. by 12 in., another 18 in. by 18 in., and the third 24 in. by 24 in. The average total dry weight production of belladonna for the two seasons and the total dry weight production of hyoscyamus in the single season were recorded in pounds per acre. The annual production in pound of dry weight per plant was also calculated and recorded. The results of this experiment are shown in Table III. The number of plants per acre shown for the different spacings allows for every seventh row to be vacant for field operations. Table III shows that both the belladonna and hyoscyamus tend to produce greatest dry weight *per plant* in the spacing of 18 in. by 18 in. although the total production of belladonna per acre was greatest in the 12-in. spacing due to the large number of plants in the close spacing. Considering hyoscyamus only, this spacing produced 745 lb. more than that of 12 in. by 12 in. in spite of having 9295 plants per acre less than the latter.

CONCLUSIONS

The results of the three studies, though not subjected to statistical analysis, indicate:

1. That belladonna plants may have a higher alkaloidal assay when fertilized with ammonium sulfate soon after establishment in the field than when fertilized at later stages of growth.

2. That belladonna plants may have a higher alkaloidal content at the flowering stage than at either an earlier or a later stage of development and hence, the best harvest time would probably be during the flowering period.

3. That the yield *per plant* in dried weight production for both belladonna and first-year hyoscyamus of the biennial variety may be expected to be higher in a plant spacing of 18 in. by 18 in. than in either a closer or a wider spacing.

4. That the total yield of belladonna in dried weight *per acre* may be expected to be higher in a 12-in. spacing than in the 18-in. (or wider) spacing, due to the greater number of plants per acre.

CORRECTION

In the article entitled, "The Spectrophotometric Determination of Methapyrilene Hydrochloride and Related Antihistaminic Derivatives of N-(2-pyridyl)-ethylenediamine," by Eric W. Martin and Joseph W. E. Harrisson, [THIS JOURNAL, **39**, 390–392 (July, 1950)] the caption "Prophenpyridamine, Trimeton (Schering)," appearing under a structural formula on page 390, should be changed to read, "Pyriphenamine derivatives under investigation."

The Potentiometric Titration of Aminophylline and Aminophylline-Phenobarbital Mixtures*,†

By ANDREW BARTILUCCI‡ and CLARENCE A. DISCHER

The literature is briefly reviewed for methods of determining aminophylline, theophylline, and phenobarbital individually and in combinations with each other. Procedures are presented for potentiometrically titrating phenobarbital, theophylline, and aminophylline; combinations of phenobarbital with either aminophylline or theophylline. Typical titration curves are presented, and the data from several titrations are tabulated. The results obtained by titrating aminophylline potentiometrically are shown to compare favorably with those obtained by the U. S. P. method. The results obtained by potentiometrically titrating mixtures of theophylline and phenobarbital were found to be compatible with the known amounts of these substances present.

THE combining of two or more organic drugs into a single dosage form has complicated the problem of analyzing medicinal preparations. While much progress has been made in this branch of pharmaceutical chemistry in the past few years, a survey of the literature failed to reveal a method for the quantitative determination of aminophylline and phenobarbital combined in tablet form. The evaluation of the methods for assaying these tablet constituents and the results of a preliminary investigation in the search for a more reliable method are the subject matter of this report.

Although the literature does not describe a method for the determination of combinations of aminophylline with phenobarbital in tablets, various methods are reported for the determination of the two compounds individually (1–9). In addition, a method for the determination of combinations of barbiturate and theobromine, an isomer of theophylline, has been reported by Bell (10). The more widely used procedures follow.

Phenobarbital.—The U. S. P. (1) extraction method using a volatile solvent frequently yields high results because of the extraction of impurities which are weighed along with the barbiturate (19).

Among the volumetric methods is the direct titration of the barbituric acid with alkali (6), and a turbidimetric method based on the fact that silver nitrate reacts with phenobarbital in alkaline media to form insoluble silver phenobarbital (7–9, 19).

Mattson and Holt (5) recently modified a colorimetric method developed by Koppanyi and his associates (3, 4), based on the color developed by the reaction of a barbiturate with a cobaltous salt in an alkaline medium. The color reaction of the cobalt-barbiturate complex is not specific and is produced by theophylline and theobromine.

Aminophylline.—Like phenobarbital, the theophylline in aminophylline may be extracted by a suitable solvent from simple preparations and assayed gravimetrically by weighing the residue (11). In a method proposed by Stevens and Wilson (12), and recognized by the U. S. P. (2), theophylline is precipitated as a silver complex with silver nitrate, the excess of that reagent being determined by back-titration. A modification of this method whereby the nitric acid which is quantitatively liberated during the complex formation is titrated with alkali, was proposed by Boie (13) for theobromine, and applied to theophylline by Schulek and Rozsa (14).

Potentiometric Methods.—Potentiometric titration of each of these alkaloids has been reported. Ogston (15), using a calomel electrode and a hydrogen electrode, measured the dissociation constants of methylated xanthines including theophylline. Payne (16) and more recently Waters, Berg, and Lachman (17) reported on the quantitative potentiometric titration of barbiturates with alkali using a glass electrode. Mattocks and Voshall (19) developed a titrimetric procedure whereby a phenobarbital preparation was titrated with silver nitrate, using a silver, silver-silver chloride electrode system.

EXPERIMENTAL

The acidic nature of both substances suggests titration with alkali. While phenobarbital had been titrated with alkali and the end point determined with a suitable indicator (6), no such method was

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found for theophylline. This may be due to the very weakly acid nature of the latter. It was decided to perform a number of potentiometric titrations on: (*a*) pure samples of each compound, and (*b*) mixtures of the two, and to examine the titration curves obtained to determine the feasibility of the method for quantitative determination of the mixed compounds.

The Leeds & Northrup model K-2 potentiometer was used in conjunction with a normal calomel electrode and a smooth platinum electrode.

The titrant was added generally in increments of 0.1 cc. near the end point. After stirring, equilibrium was reached in about fifteen seconds.

The end point was determined from the tabulated data by the method given by Kolthoff and Laitinen (18).

Procedure Used for Phenobarbital.—An accurately weighed sample of approximately 0.07 Gm. of phenobarbital was dissolved with the aid of heat in 150 cc. of distilled water. After cooling, the solution was potentiometrically titrated with 0.1 *N* NaOH.

The curve shown in Fig. 1 is typical for this potentiometric determination. Some of the determinations are summarized in Table I.

TABLE I.—POTENTIOMETRIC TITRATION OF PHENOBARBITAL

Sample Wt.	Alkali *N*	Cc. Used	Wt. Found	%
0.0614	0.0955	2.88	0.0638	104.0
0.0697	0.0955	3.21	0.0712	102.0
0.0795	0.1018	3.37	0.0796	100.0
0.0665	0.1018	2.80	0.0661	99.5

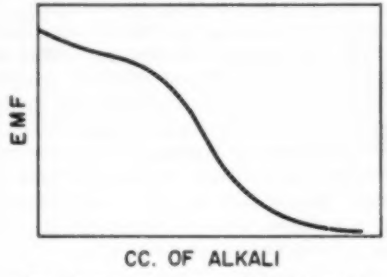

Fig. 1.—Titration curve of phenobarbital.

Procedure Used for Aminophylline.—An accurately weighed sample of approximately 0.12 Gm. of aminophylline was dissolved in 150 cc. of distilled water and potentiometrically titrated with 0.1 *N* NaOH.

The curve shown in Fig. 2 is typical of the potentiometric titration of theophylline in aminophylline. Table II compares several potentiometric determinations with results obtained by the U. S. P. method. The U. S. P. specifies that aminophylline contain from 75 to 82% of anhydrous theophylline. The close agreement between both methods is worthy of notice.

TABLE II.—COMPARISON OF THE U. S. P. AND POTENTIOMETRIC METHODS FOR QUANTITATIVELY ESTIMATING THEOPHYLLINE IN AMINOPHYLLINE

Theophylline Found by the U. S. P. Method (1), %	Theophylline Found by Potentiometric Method, %
1st detn.: 80.40	1st detn.: 80.30
2nd detn.: 80.35	2nd detn.: 80.35

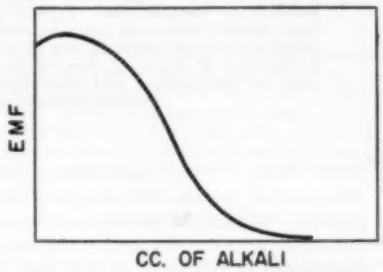

Fig. 2.—Titration curve of aminophylline.

Procedure Used for Aminophylline-Phenobarbital Mixtures.—An accurately weighed sample of approximately 0.07 Gm. of phenobarbital was dissolved in 150 cc. of distilled water with the aid of heat. After cooling, an accurately weighed sample of approximately 0.1 Gm. of aminophylline was dissolved, and the solution potentiometrically titrated with 0.1 *N* NaOH.

Since phenobarbital is more acidic than theophylline, the first end point obtained represents the phenobarbital in the sample. The volume of alkali used by the phenobarbital was subtracted from the total volume indicated by the second end point. This difference represented the volume of alkali needed to neutralize the theophylline. To ascertain the stoichiometric end point of the theophylline, the theophylline content of the aminophylline used as samples was previously determined by the U. S. P. method (2).

The results of several titrations of the mixture are recorded in Table III. The results were not considered entirely satisfactory. The weight of theophylline found was also calculated by using the amount of phenobarbital known to be present, but the same relative deviation persisted.

Procedure Used for Theophylline.—An accurately weighed sample of theophylline was dissolved in 150 cc. of distilled water with the aid of heat. After cooling, the solution was potentiometrically titrated with 0.1 *N* NaOH. Figure 3 is typical of the curve obtained.

Initial determinations of theophylline in water as a solvent were not considered satisfactory. It was observed that results obtained with increased amounts of theophylline were more consistent. An increase in the weight of sample dissolved was made feasible by using alcohol as the solvent. The use of this solvent led to results more readily interpreted than when water alone was used as a solvent.

TABLE III.—POTENTIOMETRIC TITRATION OF AMINOPHYLLINE-PHENOBARBITAL MIXTURES

	Wt. Used	Cc. Used[a]	Wt. Found	%
Phenobarbital	0.0586	2.50	0.0561	96.0
Aminophylline theophylline content	0.1279	...	...	...
	0.1028	5.86	0.1040	101.0
Phenobarbital	0.0588	2.10	0.0471	80.0
Aminophylline theophylline content	0.1364	...	...	...
	0.1096	6.00	0.1045	95.5
Phenobarbital	0.0529	2.35	0.0529	100.0
Aminophylline theophylline content	0.0898	...	...	...
	0.0722	3.88	0.0676	93.5
Phenobarbital	0.0550	2.41	0.0541	98.5
Aminophylline theophylline content	0.1076	...	...	...
	0.0864	4.47	0.0779	90.0

[a] 0.0966 *N* alkali used for each determination.

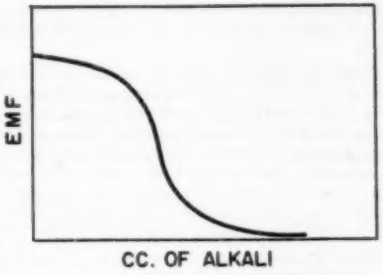

Fig. 3. Titration curve of theophylline.

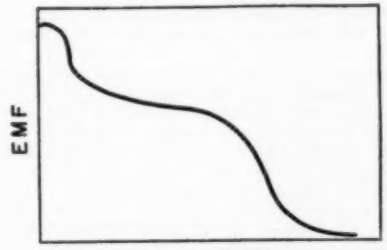

Fig. 4. Titration curve of phenobarbital and theophylline.

TABLE IV.—POTENTIOMETRIC TITRATION OF THEOPHYLLINE-PHENOBARBITAL MIXTURES

	Wt. Used	Cc. Used[a]	Wt. Found	%
Phenobarbital	0.0465	1.92	0.0454	97.5
Theophylline	0.2226	12.19	0.2233	100.5
Phenobarbital	0.0667	2.74	0.0647	97.0
Theophylline	0.1739	9.47	0.1735	100.0
Phenobarbital	0.0664	2.70	0.0638	96.0
Theophylline	0.1713	9.31	0.1706	99.5
Phenobarbital	0.0663	2.71	0.0640	96.5
Theophylline	0.2220	12.16	0.2228	100.5

[a] 0.1018 *N* alkali was used for each determination.

Procedure Used for Theophylline-Phenobarbital Mixtures.—An accurately weighed sample of approximately 0.2 Gm. of theophylline and 0.06 Gm. of phenobarbital was dissolved in 150 cc. of ethyl alcohol and potentiometrically titrated with 0.1 *N* NaOH. A blank was run on the solvent and suitable corrections made.

The curve shown in Fig. 4 is typical of those obtained by titrating the mixture. Table IV summarized the results obtained.

SUMMARY

1. The literature is briefly reviewed for methods of determining aminophylline, theophylline, and phenobarbital individually and in combinations with each other.

2. Procedures are presented for potentiometrically titrating phenobarbital, theophylline and aminophylline; combinations of phenobarbital with either aminophylline or theophylline. Typical curves are presented, and the results of several titrations are tabulated.

3. The results obtained by titrating aminophylline potentiometrically are shown to compare favorably with those obtained by the U. S. P. method.

4. The results obtained by titrating mixtures of theophylline and phenobarbital were found to be compatible with the known amount of these substances present.

Practical applications utilizing the information obtained by this preliminary investigation for commercial mixtures are being studied, and will be reported in a later paper.

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Synthesis of Local Anesthetics Derived from Cumic and 3-Nitrocumic Acids*

By J. T. BRYAN† and P. A. FOOTE‡,§

The methods of synthesis of a number of dialkylaminoalkanol esters of cumic and 3-nitrocumic acids are presented. A number of the physical constants of these compounds are included. All of these esters have local anesthetic properties. The esters of 3-nitrocumic acid showed this action to a lesser degree.

THE present study continues the work on cumic acid (*p*-isopropyl benzoic acid) which was reported on from this laboratory by Baxter and Foote (1) and Carkhuff and Gramling (2). The homologous relationship of this acid to benzoic acid suggested the possibility of preparing esters with certain amino alcohols, thereby producing local anesthetics of the procaine type. This work was further stimulated by the availability of cumic acid, a product of the oxidation of *p*-cymene which is obtained from the naval stores industry.

The cumic acid used in this investigation was of the technical grade with a melting point of 114–116°. A review of the methods of purification showed several that could be used; however, crystallization from methanol was chosen as the one most suitable for this work. Preparation of 3-nitrocumic acid was carried out by using fuming nitric acid as the nitrating agent. This method was chosen because of the short period of time which the nitrating agent was in contact with the cumic acid (about thirty to thirty-five minutes). Yields of approximately 95 per cent further justified the use of this method. After considering the possible reactions for esterification of the amino alcohols with cumic acid, the Schotten-Baumann reaction was considered the most adaptable. Thus, it became necessary to prepare the acid chlorides of cumic and 3-nitrocumic acids. This was accomplished by reacting the cumic and 3-nitrocumic acids with thionyl chloride.

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§ The authors wish to thank R. C. Palmer of the Newport Industries, Inc., Pensacola, Fla., for supplying the cumic acid for this study.

EXPERIMENTAL[1,2]

Purification of Cumic Acid.—One hundred grams of technical grade cumic acid was dissolved in 200 cc. of methanol in which it was very soluble at room temperature and produced a light yellow solution. This mixture was heated on a water bath to the boiling point of the methanol. To this boiling mixture, warm water was added until the cumic acid began to precipitate, then the precipitate was redissolved by additional heating. At this point, the mixture was set aside to crystallize. The cumic acid thus obtained was washed in a 1:10 dilution of methanol and water. After drying in an oven at 105°, a yield of 40 Gm. (m. p. 116–117°) was obtained.

Cuminoyl Chloride.—Forty grams of purified acid (m. p. 116–117°) was refluxed for two hours on a water bath with 48 Gm. of purified thionyl chloride. At the end of this time, the escaping hydrogen chloride and sulfur dioxide had completely subsided and the apparatus was arranged for downward distillation. The excess thionyl chloride was removed in this manner. The distillate, after the removal of the excess thionyl chloride, boiled at 252–253°.

Esters of Cumic Acid.—The esters, β-dimethylaminoethyl cumate, β-diethylaminoethyl cumate, γ-diethylaminopropyl cumate, were prepared by the Schotten-Baumann reaction according to the modifications of Mendlda (3). The cuminoyl chloride (0.2 mole) was reacted with the respective amino alcohols (0.1 mole) in the presence of a 10% aqueous solution of potassium hydroxide. Each ester separated from this mixture as an oil and was separated with a separatory funnel. All excess alkali was removed by washing with water. The esters were then dried over anhydrous sodium sulfate for twelve to twenty-four hours, after which they were fractionated under reduced pressure. Molecular weights were determined by cryoscopic method using nitrobenzene as a solvent. Physical characteristics of these esters are recorded in Table I.

Hydrochlorides of the Esters of Cumic Acid.—The hydrochlorides of β-dimethylaminoethyl cumate, β-diethylaminoethyl cumate, γ-diethylaminopropyl cumate, were prepared by passing dry hydrogen chloride into the respective esters using anhydrous ether as a solvent. β-Dimethylaminoethyl cumate hydrochloride and β-diethylaminoethyl cumate hydrochloride were purified by crystallization from ethanol. γ-Diethylaminopropyl cumate hydrochloride was purified by crystallization from amyl acetate. For each the per cent chlorine was determined gravimetrically by the silver method and compared to the theoretical amount (Table II).

[1] All melting points are corrected.
[2] The amino alcohols were obtained from the Eastman Kodak Company and were not further purified.

TABLE I.—ESTERS OF CUMIC ACID

	β-Dimethylaminoethyl	β-Diethylaminoethyl	γ-Diethylaminopropyl
Boiling point	160° at 8 mm.	152° at 4 mm.	185° at 8 mm.
Molar refraction	Calcd. 68.86; found 68.66	Calcd. 78.17; found 77.16	Calcd. 82.81; found 82.37
Molecular weight	Calcd. 235.04; found 231.50	Calcd. 263.17; found 262.36	Calcd. 277.18; found 275.82
Specific gravity $\frac{20°}{20°}$	1.0420	1.0218	0.9941
Refractive index n^{20}	1.5205	1.5102	1.5029
Per cent yield	40	41	47

TABLE II.—HYDROCHLORIDES OF ESTERS OF CUMIC ACID

	β-Dimethylaminoethyl	β-Diethylaminoethyl	γ-Diethylaminopropyl
Melting point	165–166°	131–132°	149–150°
Per cent chlorine	Calcd. 13.04; found 13.04	Calcd. 11.83; found 11.49	Calcd. 11.34; found 11.32
Per cent yield	84	79	70

TABLE III.—ESTERS OF 3-NITROCUMIC ACID

	β-Dimethylaminoethyl	β-Diethylaminoethyl	γ-Diethylaminopropyl
Boiling point	100° at 4 mm. (dec.)	180° at 4 mm. (dec.)	180° at 20 mm. (dec.)
Molar refraction	Calcd. 75.13; found 74.69	Calcd. 84.43; found 83.39	Calcd. 86.65; found 87.04
Molecular weight	Calcd. 280.14; found 275.21	Calcd. 308.16; found 305.80	Calcd. 322.30; found 315.62
Specific gravity $\frac{20°}{20°}$	1.1507	1.1074	1.0607
Refractive index n^{20}	1.5260	1.5148	1.4844
Per cent yield	35	33	63

3-Nitrocumic Acid.—One hundred grams of cumic acid (m. p. 116–117°) was dissolved in 500 cc. of concentrated sulfuric acid and cooled on ice. The mixture was stirred vigorously while 75 cc. of fuming nitric acid was added dropwise. Thirty minutes after the start of the reaction, the mixture was poured over crushed ice. The 3-nitrocumic acid was filtered off and purified by crystallization from ethanol, m. p. 156–157°. A yield of 97 Gm. was obtained.

3-Nitrocuminoyl Chloride.—Forty-five grams of purified thionyl chloride was added to 40 Gm. of 3-nitrocumic acid. The mixture was refluxed for two hours, and, at the end of this time, the excess thionyl chloride was removed by distillation. A yield of 35 Gm. of 3-nitrocuminoyl chloride which is a liquid at room temperature was obtained.

Esters of 3-Nitrocumic Acid.—The esters, β-dimethylaminoethyl 3-nitrocumate and β-diethylaminoethyl 3-nitrocumate, were prepared by the Schotten-Baumann reaction in the presence of 10% potassium hydroxide solution. γ-Diethylaminopropyl 3-nitrocumate was prepared by the same reaction using 3% potassium hydroxide solution. The esters were extracted from the alkali mixture with diethyl ether. The ethereal solutions were evaporated leaving behind the esters, which were then dried over anhydrous sodium sulfate. The excess amino alcohols were removed by fractionation under reduced pressure; however, the esters decomposed on prolonged heating even under reduced pressure. Molecular weights were determined by the cryoscopic method using nitrobenzene as a solvent.

Physical characteristics determined for the three esters of nitrocumic acid are reported in Table III.

SUMMARY AND CONCLUSIONS

The esters, β-dimethylaminoethyl cumate,[1] β-diethylaminoethyl cumate, and γ-diethylaminopropyl cumate, were prepared. The hydrochlorides of each of the above esters were also made.

3-Nitrocumic acid, β-dimethylaminoethyl 3-nitrocumate, β-diethylaminoethyl 3-nitrocumate, and γ-diethylaminopropyl 3-nitrocumate, were prepared. After many unsuccessful attempts to prepare the hydrochlorides of the esters of 3-nitrocumic acid, the preparation of these salts was abandoned.

The following physical constants were recorded for the esters: specific gravity, boiling point, refractive index, molar refractivity, and molecular weight. Melting point range and per cent chlorine were recorded for the hydrochlorides of the esters of cumic acid.

Evidence of local anesthesia in the lips and tongue was shown by all the esters of cumic acid; however, the esters of 3-nitrocumic acid showed this action to a lesser degree. Some of these are now being tested pharmacologically.

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Observations on the *p*H of Suspensions of Volclay Bentonite in Distilled Water and Clark-Lubs Buffer Solutions*,†

By MARTIN BARR‡ and EARL P. GUTH§

The results of the *p*H determinations of suspensions of Volclay bentonite in distilled water and Clark-Lubs buffer solutions are reported. Possible explanations for the reactions of the suspensions are discussed.

SINCE RECEIVING official recognition and acceptance in the twelfth revision of the United States Pharmacopeia, bentonite is finding wider and newer uses in pharmacy. It is because of this increasing use that new phenomena, characteristic of this claylike substance, are confronting us daily.

Bentonite is a claylike substance derived from volcanic ash, being found in many regions of the United States and other countries. There are two general forms available. One is sodium bentonite, also known as "northern" or "true" bentonite, obtainable from Wyoming and South Dakota. The other is the calcium bentonite, known as "southern" or "meta" bentonite, which is obtained from California and Texas (1). It is the sodium bentonite, available as Volclay bentonite,[1] which has the characteristics making it of particular interest for use in pharmaceuticals at the present time, and which meets the requirements for bentonite, U. S. P. XIII (2).

Volclay bentonite has as its chief mineral constituent, montmorillonite (3). Analysis of this bentonite (3) shows that it is an alumino-silicate, containing some iron, magnesia, lime, soda, potash, and other minor constituents. It possesses exchangeable metallic cations of sodium, calcium, potassium, and magnesium, sodium being available in largest quantities for exchange (4). It is this dominance of available exchangeable sodium cations that gives to Volclay bentonite many of its characteristic properties.

It has been observed repeatedly by the authors that the buffering of Volclay bentonite on the acid side is not a practical matter. Attempts to prepare buffered Volclay bentonite ointment bases, on the acid side, utilizing the Clark-Lubs buffers of the U. S. P. XIII, met with little success. Therefore, it was decided to study the effect of concentration of Volclay bentonite on the *p*H of its suspensions using the Clark-Lubs buffers and distilled water as the suspending mediums, in an attempt to explain the behavior of Volclay bentonite in these suspensions through interpretation of the data obtained. It is with this study that this paper is concerned.

EXPERIMENTAL

Bentonite[2] suspensions (1, 3, 5, 10, 15, 20, 25, and 30% *w/w*) were prepared by adding the bentonite to the Clark-Lubs buffer solutions (*p*H 1, 2, 3, 4, 5, 6, and 7), (5), in a mechanical mixer[3] operated at a slow rate of speed. The total amount of bentonite required for each suspension was added, in divided portions, in exactly two minutes, and the suspensions were stirred at the same rate of speed for eight minutes more. One hundred grams of each suspension was prepared and stored in 4-oz. amber jars.

Bentonite suspensions using distilled water as the suspending medium were prepared using the same procedure as described when the Clark-Lubs buffers were used as suspending mediums.

The *p*H[4] of the bentonite suspensions, buffer solutions, and distilled water were determined at the time of preparation and one, seven, and twenty-eight days after preparation. All the suspensions were stirred at a rapid rate of speed in a mechanical mixer for exactly two minutes before the *p*H determinations were made.

The results of the *p*H determinations of the suspensions at the time of preparation are recorded in Fig. 1. The recorded results of the *p*H determinations of the suspensions one, seven, and twenty-eight days after preparation are not listed since the changes were not significant.

DISCUSSION

Stevens (6), among others, has found that when various alumino-silicates are ground under water, they hydrolyze to give characteristic *p*H values. The results of the *p*H determinations of suspensions of Volclay bentonite in distilled water show that the

* Received April 21, 1950, from the Pharmaceutical Research Laboratory, College of Pharmacy, The Ohio State University, Columbus.

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[1] Available in various grades from the American Colloid Co.

[2] BC Volclay Micron Bentonite of the American Colloid Co. utilized hereon in this study.

[3] Number one speed on Hamilton-Beach Mixer, Model E.

[4] *p*H determinations were made with a Beckman *p*H Meter (Industrial Model).

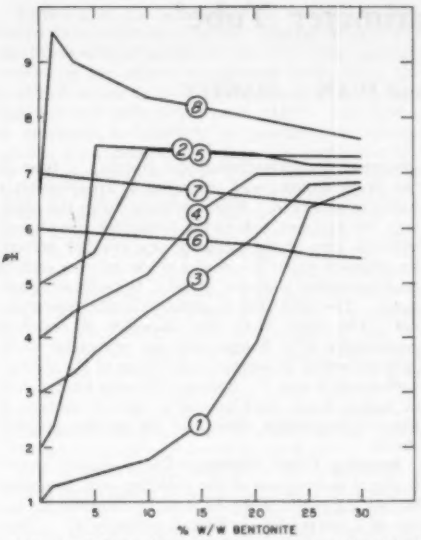

Fig. 1.—Effect of per cent bentonite on the *p*H of bentonite suspensions at time of preparation, using Clark-Lubs buffers and distilled water as suspending mediums.

Code: *1*, 1.00; *2*, 2.00; *3*, 3.00; *4*, 4.00; *5*, 5.00; *6*, 6.00; *7*, 7.00 (Clark-Lubs buffers *p*H). 8, distilled water (*p*H 6.70).

*p*H of these bentonite suspensions are alkaline. It is probable that this phenomenon is principally one of hydrolysis. It has been previously mentioned that Volclay bentonite is chiefly a silicate containing sodium as its dominant exchangeable cation. Therefore, the bentonite may be represented as Na_2SiO_3 for explanation purposes. The hydrolysis reaction may then be illustrated as:

$$Na_2SiO_3 + 2HOH \rightleftharpoons H_2SiO_3 + 2NaOH$$

The silicic acid formed has little effect on *p*H as it is weakly ionized. NaOH is a strong base, however, and since it is almost completely ionized the solution thus produced is alkaline. Thus suspensions of Volclay bentonite are alkaline.

It has been shown in Fig. 1 that the *p*H of Volclay bentonite suspensions, using distilled water as the suspending medium, falls as the concentration of bentonite is increased. This is in agreement with the report of Buehrer and Williams (7). As yet, no explanation can be given as to the cause of this fall in *p*H. It is possibly due to an electrolyte effect on the bentonite, but more research is needed before any conclusions may be reached.

The results in Fig. 1 have also shown that Clark-Lubs buffer solutions of the U. S. P. XIII are not capable of maintaining their original *p*H upon addition of Volclay bentonite. This may be due to the cation exchange phenomenon which is exhibited by clay materials (8). Bentonite has the property of exchanging, stoichiometrically, its exchangeable cations for other cations in water solution. Therefore, since hydrogen is a very potent replacing cation (9), it would not be expected that the buffer solution would maintain its original hydrogen-ion concentration in the presence of Volclay bentonite.

When Clark-Lubs buffer, *p*H 1.00, was used as the suspending medium, there was an exchange between the hydrogen ions in solution and the alkaline cations of the Volclay bentonite. The resultant rise in *p*H may be attributed to two causes: (*a*) The bentonite may take hydrogen ions from the buffer solution, thus reducing the hydrogen ion concentration in solution, and (*b*) the bentonite releases alkaline cations resulting in formation of alkaline hydroxides, which likewise produce a decrease in concentration of hydrogen ions. When Clark-Lubs buffer, *p*H 2.00, was used as the suspending medium, the rise in *p*H occurred with smaller amounts of bentonite than was the case with the buffer of *p*H 1.00, because the amount of 0.2 *M* HCl in the latter buffer solution is much less than that of the *p*H 1.00 buffer solution. The same phenomenon may be responsible for the rise in *p*H of the bentonite suspensions when Clark-Lubs buffers, *p*H 3.00, 4.00, and 5.00 were used as suspending mediums. When buffer solutions of *p*H 6.00 and 7.00 were used as the suspending mediums, there was some buffering activity indicated, with a slight fall in *p*H being evidenced as the concentration of bentonite was increased.

Clark-Lubs buffers of increased buffer capacity were prepared, and these buffering solutions were used as suspending mediums for the Volclay bentonite suspensions. Because of the increased buffer capacity of these solutions, the bentonite was less detrimental in producing a decrease in hydrogen-ion concentration; however, these concentrated buffers could not be considered adequate buffers for Volclay bentonite.

A study of the effect of time on the *p*H of the Volclay bentonite suspensions showed negligible variation over a period of twenty-eight days. It has been reported that cation exchange reactions in clays are instantaneous. However, these reactions are not necessarily complete, therefore the slight change in *p*H of the bentonite suspensions over a period of time is probably due to completion of the reactions.

SUMMARY

1. Suspensions of Volclay bentonite in distilled water are alkaline by hydrolysis. The *p*H of these suspensions falls as the bentonite to water ratio increases.

2. Volclay bentonite is resistant to acid buffering with the Clark-Lubs buffers because of the cation exchange property of the bentonite.

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A Capillary Polarimeter Tube*

By FRANK M. GOYAN and IVAN J. SZEKELY

A technical description of a new capillary polarimeter tube and of several methods for securing cover glasses is given. The new tube has been used for precision measurements on sucrose, using a wire recorder to take data in a darkroom; for work with essential oils; and for a novel "optical titration."

IN CONNECTION with the polarimetry of penicillin solutions, one of the authors had occasion to design a capillary polarimeter tube that could be filled and emptied with a medicine dropper. A survey of the literature showed a need for such a tube. The Fischer capillary tube (1) is not easy to fill, and Békésy's tube (2, 3) seems to require elaborate procedures both in construction and in filling. Smith and Ehrhardt (4) insert a suitable thick-walled capillary inside a partially filled standard tube, thereby limiting the sample to the bore of the capillary plus a small residual volume. Naumann (5) reports that the etched inner surface of his black glass tube eliminates optical flare. However, this flare or "halo" caused by light reflected from the inner surface of the capillary has not proved to be a serious handicap with the tube described below because suitable diaphragms are introduced into the optical path.

DESCRIPTION

Six of the glass units represented by *D* of Fig. 1 were produced commercially[1] from 12-mm. o. d. capillary having an inside diameter of 2.6 mm. and an approximate working volume of 1.5 cc. Liquid trapped at the extreme ends of the capillary beyond the side tubes is rapidly and automatically mixed during filling, and air bubbles are effectively removed from this area by tapping. However, it is desirable to minimize the distance between the entrance of the side tube and the cover glass.

The assembly shown in Fig. 1 consists of the glass tube *D* and its brass holder. The holder is designed to secure any of the interchangeable tubes in optical alignment in a polarimeter. Tubes are held by spring *E* in a semicircular trough soldered to rod *A*. The trough is made from brass tubing and provided with two adjusting screws *F* which bear against the capillary, providing fine adjustment needed to obtain exact optical alignment of several different tubes having slight external irregularities. Initial alignment is accomplished by securing a tube in the brass trough, and centering it approximately with the screws *F*. While sighting down the capillary, the centered hole in the brass disk *C* is aligned with the bore of the capillary, and the disk secured by means of screw *B*. A wax or low melting cement may be applied between *A* and *C* to facilitate alignment. The other disk is adjusted in the same manner. The disks have the diameter of standard polarimeter tube flanges and are supported vertically by virtue of accurate machining of the contact surfaces of *A* and *C*. Spring *E* is made from a strip of spring brass bent around a slotted section of brass tubing which slides into the holding position shown.

Securing Cover Glasses.—Cover glasses shown in Fig. 1 at each end of the capillary were produced by cutting round disks 12 mm. in diameter by the use of a slotted brass "biscuit cutter" (6). These disks may be sealed in place with a variety of agents or held by small coil springs stretched between flat metal end rings and metal collars tightened around the main capillary about 3 cm. from each end. This detail is not shown in Fig. 1 because it has not been used extensively.

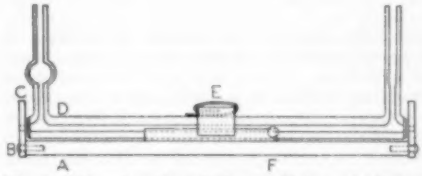

Fig. 1.—Line drawing of the capillary polarimeter tube and holder.

The original tube made in this laboratory has been used successfully for hundreds of different samples using cover glasses cemented in place with DeKhotinsky cement (7) which presents the difficulty of heating the cover glasses. Several plastic cements such as polystyrene "coil dope," "Duco cement," etc., were used with limited success until the problem of applying heat was solved by modifying an electric soldering iron to provide, in place of the soldering point, a flat surface larger than the cover glasses. By means of this device supported above table level, it is easy to apply heat by bringing the end of a tube with a cover glass in place up to the hot surface. The temperature of the surface is adjusted with the aid of a variable transformer, and determined roughly by observing the melting behavior of solder, tin, bismuth, and lead.

Glycol phthalate (7), made by refluxing equivalent amounts of glycol and phthalic anhydride for approximately twenty-four hours, is excellent for tubes to be used below 30° because it may be used like DeKhotinsky but at a lower temperature.

* Received May 5, 1950, from the University of California, College of Pharmacy, The Medical Center, San Francisco.
Presented to the Scientific Section, A. PH. A., Atlantic City meeting, May, 1950.

[1] O. C. Rudolph & Sons, Caldwell, N. J.

Butvar ("H. V." Monsanto) and shellac were also found to be effective cements. Shellac dissolved in methyl alcohol is applied to the ends of the capillary tubes and the solvent evaporated before using the modified soldering iron which is heated to a temperature just sufficient to melt solder. The shellac is inspected at intervals to prevent overheating. During each inspection, pressure is applied evenly to the cover glass with metal forceps. Butvar seals are made in the same manner except that a 7% solution of the plastic in a solvent consisting of two parts ethyl alcohol and one part acetone is used, and the temperature of the modified soldering iron is raised to the melting point of metallic bismuth. Only in one case with very thin Butvar films was strain detected in the cover glasses by observation in the polarimeter. The thicknesses of the individual Butvar, glycol phthalate, and shellac films were found to range from less than 5 μ to 15 μ as determined with an improvised micrometer device. Excess plastic may be removed from the optical surfaces by allowing acetone to stand in the tubes, followed by water and hot cleaning solution.

Butvar seals are superior from the standpoint of strength and ability to withstand high temperatures. However, both alcohol and acetone dissolve Butvar, causing gradual removal of the plastic around the point of contact with solvent. Very thin clear Butvar seals, however, are able to hold alcohol for twenty-four hours or longer. Organic solvents are probably better managed with aid of the spring cover glass holder described above, either with or without the use of silicon stopcock grease, depending upon fit and upon the length of time the solvent must be retained.

Filling and Rinsing.—Special medicine droppers with elongated tips are used for adding and withdrawing samples. Sheet rubber disks may be fitted on the side tubes to prevent drops of liquid from accidentally running down the outside surfaces into the optical path. Mixing of a sample already in the tube is accomplished by tilting several times so that the sample flows in and out of the glass bulb. Conventional methods of washing, drying, and refilling necessitate swinging and tapping to remove air bubbles trapped near the ends.

Systematic rinsing experiments using radioactive sodium phosphate solutions (P^{32}, half-life 14.5 days) were made using the original tube. This work was done at the suggestion of Dr. J. J. Eiler, and with the help of Dr. Kwan-Hua Lee. Similar experiments were tried with concentrated dye solutions and with sucrose solutions, using appropriate methods of analysis in each case. The amount of original solution retained in the tube and carried into the first rinse water varied from about 3 to 5%, indicating the desirability of discarding the second filling. In the above experiments, each filling was removed as well as possible with a medicine dropper.

Precision Polarimetry with the Capillary Tube.—One of the authors made careful measurements on sucrose C. P. (Pfanstiehl Chemical Co.) using the O. C. Rudolph polarimeter (Model 80). The work was done at 24° using a filtered sodium vapor arc as the light source. For each value taken, ten independent readings were spoken into a wire recorder and later averaged on a calculator. Table I gives the summary of results observed and calculated. The calculations are based on classical methods (8) which are in agreement, within the limits of accuracy required for this study, with the more recent developments reported by the National Bureau of Standards (9).

TABLE I.—OBSERVED AND CALCULATED VALUES FOR THE SPECIFIC ROTATION OF SUCROSE

Weight Per Cent of Sucrose[a]	Observed Angle of Rotation[b]	Specific Rotation (Observed) 24°	Specific Rotation (Calcd.)[c] 24°
1.202_7	1.5968	66.26	66.37
1.202_7	1.5980	66.33	66.37
1.208_8	1.3680	66.40	66.37
1.208_8	1.3695	66.48	66.37
0.4266	0.5640	66.10	66.35
0.4266	0.5633	66.04	66.35
0.3044	0.4020	66.07	66.35
0.3044	0.4025	66.16	66.35

[a] This column may also be read as the approximate minimum weight of sucrose in centigrams required for the reading.

[b] Each value is an average of ten readings each read to 0.002°.

[c] The equations used and the relative density values at 20° are taken from Browne and Zerban (8). The calculated relative densities (d_l) for the sucrose solutions in decreasing order of concentration were: 1.0019, 1.0012, 0.9998, and 0.9993.

The results shown in Table I indicate that the largest divergence is within the accuracy limits of observation. For solutions of about 1% concentration, the average deviation is within 0.1%. On the more dilute solutions the accuracy of observation is limited by the polarimeter to about 0.4%. The largest divergence for those solutions does not exceed 0.4%.

At the suggestion of H. D. Bryan, University of Nebraska, College of Pharmacy, a few readings were made using essential oils. The results were successful, showing that visibility is not sacrificed with colorless or nearly colorless liquids of high refractive index.

Optical Titrations.—In order to demonstrate the value of the tube for the study of chemical reactions involving changes in rotation, ephedrine hydrochloride was "titrated" against NaOH in the tube without removing the solution. The technique involves adding small uniform samples of reagent from a micro-pipette, mixing, and reading the polarimeter after each addition. The polarimeter readings are plotted as a function of equivalents of NaOH added to give typical smooth curves.

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Book Reviews

The Condensed Chemical Dictionary. 4th ed. FRANCIS M. TURNER, Editorial Director. Revised and enlarged by ARTHUR and ELIZABETH ROSE. Reinhold Publishing Corporation, New York, 1950. xxix + 726 pp. 16 x 23.5 cm. Price $10.

Those who have used one of the previous (1919, 1930, or 1942) editions of this dictionary will be pleased to know that a new, enlarged, and greatly improved fourth edition is now available. Anyone having use for a chemical dictionary designed to serve the needs of professional chemists and pharmacists as well as people not formally educated along chemical lines will find this book extremely useful. The new fourth edition has been completely revised and enlarged with the addition of 5,000 new items, making a total of over 23,000 entries. Many chemical specialties sold under registered trade mark names or names otherwise protected are listed. A list of 400 manufacturers of these chemical specialties is included and referred to by number in each entry involved. This feature is especially useful in locating sources of chemicals known only by proprietary names. Cross references are used exclusively to assist in locating quickly-wanted information. The useful information on containers, shipping regulations, and safety instructions has been continued and brought up-to-date. The reviewer is of the opinion that the entries to the new fourth edition of *The Condensed Chemical Dictionary* have, for the most part, been well chosen and that the book can be recommended to those who need quick and accurate information on chemical and related terms.

Melting and Solidification of Fats. By ALTON E. BAILEY. Interscience Publishers, Inc., New York, 1950. xiv + 357 pp. Illustrated. 15.5 x 23.5 cm. Price $7.

This monograph was prepared for the purpose of collecting all available material on the subject and correlating and interpreting these factors so as "to reveal the interrelationships between different phenomena associated with phase transformation, as well as the systematic variations that occur in specific properties of fats with similar variations in their molecular structure." The book represents an especially complete and well-organized survey of physicochemical concepts of melting and solidification of fats singly and in combinations.

The subject is introduced by a general section which describes the structure, size, and shape of fat molecules and describes briefly the concepts of dipoles, hydrogen bonding, van der Waals' forces, etc. Crystal structure is considered in some detail and there is an unusually good section on melting theories and on phase diagrams. A very useful chapter follows which describes in considerable detail the laboratory technique involved in various measurements that are used for specific tests, as well as preparation of phase diagrams. Pure compounds are then discussed with respect to their melting and solidification and the physical phenomena associated with these transitions.

The author makes the point that dissolving of a substance is a special case of mixed melting and then proceeds to treat in considerable detail the solubility of fatty acids and fats. The latter portion of the book is devoted to practical applications of previously discussed physicochemical concepts and points out, in masterful fashion, deductions that may be made from these data. The book cites a very large number of references, these references being placed at the bottom of the pages for greatest convenience.

There is no question but what the author has achieved a tremendous task and has succeeded in presenting an unusually valuable reference on fats that will have daily usefulness for most workers in this field.

The Biochemistry of B Vitamins. By ROGER J. WILLIAMS, ROBERT E. EAKIN, ERNEST BEERSTECHER, JR., and WILLIAM SHIVE. Reinhold Publishing Corporation, New York, 1950. x + 741 pp. 15.5 x 23.5 cm. Price $10.

This is the latest edition to the list of American Chemical Society monographs, and its subject is one well suited to such treatment, for certainly the information that has accumulated on the B vitamins has become so large and complex that this monograph will be most useful.

The authors had in mind at the writing of this book to survey the chemistry of the B vitamins and to present the material in such a form that it will be understandable to workers in other fields, and will serve to introduce to the field those chemists who so frequently nowadays find themselves unexpectedly involved in nutritional chemistry. The authors also wished to present a survey of progress that would be useful to students by serving to bring their knowledge up-to-date in this particular field. Having read the book, one cannot help but admire the skillful way in which the authors have realized their objectives.

This monograph is divided into four major sections: A—Characterization, Distribution, Assay, and Biogenesis of B Vitamins; B—Catalytic Functions of the B Vitamins; C—Role of the B Vitamins in Animal and Plant Organisms; D—Comparative Biological Activities of the B Vitamins and Related Compounds. The reader finds this arrangement logical, orderly, and conducive to clear understanding.

There is included an historical introduction which contains some general statements as to the occurrence of the B vitamins and an attempt at definition of these factors. Following this is a brief chapter

which presents the chemical structures of the members of the vitamin B family. References are made to reviews and individual papers which give the detailed chemistry of these materials.

There is a chapter on the occurrence of these vitamins, both in plant and animal tissues, and another chapter presents descriptions of the method of assay. Useful comments are made, particularly with respect to special treatment that is required when assaying for vitamin content of particular mixtures or natural products. Included in this chapter are chemical, microbiological, and biological assay methods. A very interesting section on the biogenesis of B vitamins will appeal particularly to the academic mind. A section devoted to biochemical reactions and their catalysts classifies the types of reaction and explains the role of the vitamin involved as a catalyst. The importance of understanding the chemical processes involving vitamins is well illustrated. The book progresses logically to the metabolic functions of the B vitamins, reviews the methods of assessing vitamin B requirements and factors influencing these requirements, such factors as age, sex, occupation, and individual variations being considered. The metabolism of B vitamins is discussed, and knowledge as to the absorption, excretion, and conditions affecting these processes. Pharmacology and toxicology of the B vitamins are considered, and there is a large section on deficiency states.

Before progressing to the details of the use of enzyme reactions and inhibition studies serving to clarify biochemical reactions of B vitamins, a very good introduction to the fundamentals of enzyme reactions is presented. The inhibitors of the various B factors are considered in some detail, this section including a large number of antivitamin substances.

This book presents in clear and readable form a very large amount of information and will certainly be of great value to anyone concerned with the vitamin B group.

Physico-Chemical Constants of Pure Organic Compounds. By J. TIMMERMANS. Elsevier Publishing Company, Inc., New York, 1950. viii + 693 pp. 17 x 24.5 cm. Price $12.50.

The object of this book is to record "as completely as possible those physico-chemical constants of organic compounds which have been measured with sufficient care to warrant their acceptance as data established with a precision worthy of contemporary science." This object limits considerably the number of compounds that are listed, since thousands of compounds do not meet this criterion for various reasons: the literature may not give a sufficient description of the methods used so that accuracy of determinations and purity of the material could be assured; also, the constants of a great majority of compounds have been determined only with sufficient accuracy to prove the identity of the chemical. Rather stringent limitations are placed on the various physical constants which were accepted. For example, a boiling range would not be accepted which exceeded one-tenth degree. It is this type of careful scrutinization of literature values which makes the book outstanding and useful, for one can feel that information obtained from this source will be reliable. It is also of value that literature sources are given for the data contained in the book, references being made both to methods of purification and to determinations of constants.

Chemicals are arranged in a logical fashion: hydrocarbons, halogenated derivatives, oxygenated derivatives, nitrogen compounds, mixed compounds (such as nitrohaloalkanes), sulfur derivatives, phosphorous derivatives, etc.

Constants include critical constants, densities of liquid and saturated vapor, rectilinear diameter, saturated vapor pressure, boiling point, freezing point, the various constants of state, and heat constants. All these values are given so that there is uncertainty only in the last figure.

Material is easy to locate in the book, since there are both subject and formula indexes.

This promises to be a valuable reference work, and it is hoped that the book will be revised regularly as new data is made available.

Industrial Chemicals. By W. L. FAITH, DONALD B. KEYES, and RONALD L. CLARK. John Wiley and Sons, New York, 1950. xi + 652 pp. Illustrated. 15 x 23.5 cm. Price $8.

This book presents in a single volume the compilation of frequently-needed technical and economic data relating to 106 industrial chemicals most widely used as intermediates in the chemical and pharmaceutical industries. Information of the type recorded relative to these chemicals has heretofore been difficult to obtain because it has so often been widely diffused in numerous books, magazines, and pamphlets. The design of *Industrial Chemicals* has produced a very convenient reference book. Each chemical is covered in a separate monograph, and the data are presented in the same order in each monograph. The data on each chemical are presented in the following order:

1. Name and chemical formula.
2. Manufacturing processes in current use, including simplified flow sheets.
3. Equations for the principal reactions involved and average yield expectation.
4. Raw material requirements per unit of commercial product. In some cases, utility and labor requirements are shown.
5. Graph showing production for the past twenty years.
6. Generalized use pattern.
7. Graph showing price variation for the past twenty years.
8. Miscellaneous data, including physical properties, commercial grades, shipping regulations, and usual containers.
9. Economic aspects, generally including historical data, a discussion of competitive products and processes, recent trends in manufacturing and sales, actual and economic plant size, and approximate plant cost.
10. A map showing the location of manufacturing facilities in the United States.

Information of the type presented in *Industrial Chemicals* should be of special value to those engaged in the manufacture and sale of chemicals and pharmaceuticals and should serve as a useful reference book for teachers and students of subjects relating to chemical and pharmaceutical processes and manufacturing.

1951 Iodine Research Award Nominations Requested

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1951 Chilean Iodine Educational Bureau Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine. Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to Robert P. Fischelis, Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1951 Award, nominations must be received on or before January 1, 1951.

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During the period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Chilean Iodine Educational Bureau, Inc.

The award consists of $1000 and a diploma setting forth the reasons for selection of the recipient. It may be presented annually at the annual meeting of the ASSOCIATION.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. In addition to the sum of the award, the recipient will receive an allowance of not more than $250 to defray his expenses in attending the meeting.

The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION'S Council. The present committee includes Justin L. Powers, chairman; Louis Gershenfeld, Charles O. Wilson, Harvey B. Haag, Heber W. Youngken, John C. Krantz, and Frank O. Taylor.

The award is now in its fourth year. The recipient of the first award was Dr. William T. Salter, Yale University pharmacologist, and the second recipient was Dr. George M. Curtis, Chairman of the Department of Surgical Research and Professor of Surgery at Ohio State University.

Dr. C P. Leblond, Professor of Anatomy, McGill University, Montreal, Canada, will receive the third award and will give the 1951 Iodine Award lecture.

FIRST SUPPLEMENT TO N. F. IX

The text of the First Supplement to N. F. IX appears on the next four pages of this issue of the Scientific Edition of THIS JOURNAL. The Supplement will also be published in the same relative position in the November issue of the Practical Pharmacy Edition of the JOURNAL. The four pages, torn from either edition of THIS JOURNAL, constitute an official copy of the First Supplement to N. F. IX. Reprints of this Supplement will be sent in due time to owners of copies of N. F. IX who have filled out and mailed the card provided on the back flyleaf of each book. Those who have not returned the card are urged to do so promptly if they wish to receive reprints in addition to the copies of the First Supplement to N. F. IX published in both editions of THIS JOURNAL.

FIRST SUPPLEMENT TO THE
NATIONAL FORMULARY
NINTH EDITION

N. F. IX

OFFICIAL FROM NOVEMBER 1, 1950

By virtue of the authority conveyed in Chapter III, Article VI and Chapter VIII, Article V of the By-Laws of the American Pharmaceutical Association, the Council of the Association appointed the Committee on National Formulary, approved the text of this Supplement prepared by the Committee, authorized the printing and distribution thereof, and fixed November 1, 1950, as the date upon which the First Supplement to the National Formulary, Ninth Edition, shall become official.

ROBERT P. FISCHELIS, *Secretary*
Council of the A. PH. A.

JUSTIN L. POWERS, *Chairman*
Committee on National Formulary

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ADDITIONS, CHANGES, AND CORRECTIONS

Notice Concerning Patent or Trade-Mark Rights

The inclusion in the Ninth Edition of the National Formulary of any drug in respect to which patent or trade-mark rights may exist shall not be deemed, and is not intended as, a grant of, or authority to exercise, any right or privilege protected by such patent or trade-mark. All such rights and privileges are vested in the patent or trade-mark owner, and no other person may exercise the same without express permission, authority, or license secured from such owner.

The page numbers cited refer to N. F. IX.

Calcium Glycerophosphate, page 107—Change the first paragraph in the monograph to read:

Calcium Glycerophosphate is the normal calcium salt of glycerophosphoric acid and, when dried to constant weight at 130°, it contains not less than 98 per cent of $C_3H_7CaPO_6$.

Change the paragraph entitled *Loss on drying* to read:

Loss on drying—Dry Calcium Glycerophosphate at 130° to constant weight: it loses not more than 12 per cent of its weight, page 741.

Change the first sentence of the *Assay* to read:

Dry about 400 mg. of Calcium Glycerophosphate at 130° to constant weight, weigh accurately, dissolve in 100 cc. of water and add 1 cc. of hydrochloric acid.

Ferric Glycerophosphate, page 210—Change the first paragraph in the monograph to read:

Ferric Glycerophosphate, dried at 130° to constant weight, contains not less than 17 per cent of Fe, corresponding to not less than 95 per cent of $C_9H_{21}Fe_2O_{18}P_3$.

Change the paragraph entitled *Loss on drying* to read:

Loss on drying—Dry Ferric Glycerophosphate at 130° to constant weight: it loses not more than 12 per cent of its weight, page 741.

Change the first sentence of the *Assay* to read:

Dissolve about 1 Gm. of Ferric Glycerophosphate, dried at 130° to constant weight and accurately weighed, in 25 cc. of water in a glass-stoppered flask.

Histidine Monohydrochloride Injection, page 247—Change the paragraph entitled *Histamine,* page 248, to read:

Histamine—Histidine Monohydrochloride Injection, when diluted with physiological salt solution to a concentration of 10 mg. per cc., and tested as for *Histamine* under *Histidine Monohydrochloride,* page 247, shows no greater blood pressure depressant effect than 0.2 microgram per Kg. of histamine base.

Manganese Glycerophosphate, page 311—Change the first paragraph in the monograph to read:

Manganese Glycerophosphate, dried at 110° to constant weight, contains not less than 98 per cent of $C_3H_7MnO_6P$.

Change the paragraph entitled *Loss on drying* to read:

Loss on drying—Dry Manganese Glycerophosphate at 110° to constant weight: it loses not more than 12 per cent of its weight, page 741.

Change the fourth sentence in the paragraph entitled *Lead* to read:

A 10-cc. portion of this solution contains no more than 5 micrograms of lead (corresponding to 50 parts per million) when tested according to the *Lead limit test*, page 739, using 10 cc. of ammonium citrate solution, 1 cc. of potassium cyanide solution, and 0.5 cc. of hydroxylamine hydrochloride solution.

Change the first sentence of the *Assay*, page 312, to read:

Dissolve about 1 Gm. of Manganese Glycerophosphate, dried at 110° to constant weight and accurately weighed, in 1.5 cc. of nitric acid and 5 cc. of warm water.

Potassium Hypophosphite, page 403—Change the molecular formula where it first appears in the monograph, to read: KPH_2O_2.

Resorcinol Ointment, Compound, page 430—Until further notice, the formula and directions for preparation may be changed to read:

Resorcinol	**60 Gm.**
Zinc Oxide	**60 Gm.**
Bismuth Subnitrate	**60 Gm.**
Juniper Tar	**20 Gm.**
Yellow Wax	**100 Gm.**
Petrolatum	**290 Gm.**
Wool Fat	**280 Gm.**
Glycerin	**130 Gm.**
To make	**1000 Gm.**

Melt the yellow wax and wool fat in a dish on a water bath. Triturate the zinc oxide and bismuth subnitrate with the petrolatum until smooth, and add it to the melted mixture. Dissolve the resorcinol in the glycerin; incorporate the solution with the warm mixture just prepared; then add the juniper tar, and stir the Ointment until it congeals.

Scarlet Red, page 449—Change the second sentence in the paragraph entitled *Absorption characteristics*, to read:

The absorption ratio (A at 500 mμ over A at 520 mμ) is not less than 0.83 and not more than 0.97.

Scarlet Red Sulfonate, page 450—Change the paragraph entitled *Absorption characteristics*, page 451, to read:

Absorption characteristics—Determine the absorbancies, A, of a solution containing about 5 mg. per liter of Scarlet Red Sulfonate contained in a 1-cm. absorption cell at 490 mμ and 510 mμ. The absorption ratio (A at 490 mμ over A at 510 mμ) is not less than 0.78 and not more than 0.94.

Sodium Alginate, page 468—Change the paragraph entitled *Lead*, page 469, to read:

Lead—Add 1 Gm. of Sodium Alginate to 20 cc. of nitric acid in a 250-cc. Erlenmeyer flask, and proceed as directed in the test for *Lead* under *Pectin*, page 369, beginning with the words: ". . . mix well, . . ." Sodium Alginate contains not more than 10 parts per million when tested according to the *Lead limit test*, page 739.

Thimerosal Ointment, page 546—Change the formula to read:

Thimerosal	1 Gm.
Distilled Water	10 cc.
Cholesterol	10 Gm.
Wool Fat	150 Gm.
White Wax	80 Gm.
Stearyl Alcohol	30 Gm.
White Petrolatum, a sufficient quantity,	
To make	1000 Gm.

Undecylenic Acid Ointment, Compound, page 562—NOTE: U. S. Patent 2,510,946, dated June 13, 1950, restricts the right to manufacture this Ointment.

Zinc Undecylenate, page 576—NOTE: U. S. Patent 2,511,032, dated June 13, 1950, restricts the incorporation of Zinc Undecylenate in external preparations.

Injections, page 732—Notice concerning paragraph entitled *Number of Doses in Container*, page 738.

By action of the Committee on National Formulary and the Council of the American Pharmaceutical Association the section entitled *Number of Doses in Container*, page 738 of N. F. IX, will not become effective until July 1, 1951. This postponement is to permit completion of studies into the need for exemptions in the case of certain National Formulary injections. Every effort will be made to finish the study in ample time prior to July 1, 1951, so that necessary labeling and packaging changes can be made.

Readily Carbonizable Substances, page 757—Change the parts of distilled water specified for the preparation of Matching Fluid B, in the paragraph entitled *Preparation of the Matching Fluids*, from 3.5 to 8.5.

The Dollars and Sense of RUTIN

Have you overlooked the profit possibility of adding a Rutin specialty to your line?

The market for products containing Rutin is gradually becoming larger and larger as clinical tests indicate the wider use of this important drug. The profit possibility of improving an item in a line through the use of Rutin, or introducing a new Rutin specialty, is attracting the attention of alert pharmaceutical manufacturing concerns.

Originally Rutin was largely used in the treatment of capillary fragility associated with hypertension. It has been estimated that there are in this country around 5 or 6 million people who have hypertension.

In this group there is evidence of widespread incidence of increased capillary fragility.

Further clinical tests have shown that Rutin is constantly proving valuable in the treatment of hemorrhagic diseases – opening up new and enlarged opportunities for products that contain Rutin.

Rutin (Baker) is a yellow microcrystalline, stable powder containing less than 0.002% of chlorophyll. It meets all the standards proposed by the National Formulary.

Round out your line of drug products with a Rutin specialty for added profit. For free Rutin bulletin on the Chemistry, Pharmacology, Clinical tests and uses of this important drug, write Fine Chemical Division, J. T. Baker Chemical Co., Phillipsburg, New Jersey.

Baker CHEMICAL

Baker Chemicals

REAGENT • FINE • INDUSTRIAL

Standardize On PYREX Brand Bottles

FOR LONGER SERVICE LIFE

Bottles used for preparation, storage or shipment of pharmaceuticals and antibiotics can be an expensive item if they break easily, are attacked by contents or cannot withstand repeated sterilization. That is why it will pay you to standardize on tough, durable PYREX Brand Bottles.

Made from glass No. 7740, PYREX Brand Bottles are unusually resistant to physical and thermal shock. Repeated sterilization is possible. Chemically inert and resistant to chemical attack, there is no danger of product loss or contamination through change in pH. Long service life is assured.

Especially designed for pharmaceutical operations, PYREX Brand Bottles are available in standard sizes ranging from 60 ml to 12 gallons. Bottles can be made for special purposes such as intravenous preparations. Let us know your requirements. Your inquiries will be promptly handled.

CORNING GLASS WORKS **CORNING, N. Y.**

Corning means research in Glass

Technical Products Division: Laboratory Glassware, Glass Pipe, Plant Equipment, Lightingware, Signalware, Gauge Glasses, Optical Glass, Glass Components